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Time-resolved diffuse optical tomography for non-invasive flap viability assessment: pre-clinical tests on rats

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ABSTRACT

We present a new setup for time-resolved diffuse optical tomography based on multiple source-detector acquisitions analysed by means of the Mellin-Laplace transform. The proposed setup has been used to perform pre-clinical measurements on rats in order to show its suitability for non-invasive assessment of flap viability.

Keywords: Image reconstruction techniques; Light propagation in tissues; Medical optics instrumentation; Optical diagnostics for medicine; Time-resolved imaging; Tomography

1. INTRODUCTION

In reconstructive surgery anatomical defects are often restored using autologous tissues ("flaps"). A flap includes the harvested tissues (e.g. skin, muscle, bone, etc. alone or combined) together with the connected arteries and veins and its thickness can range from few mm up to 3 cm (buried flap). When a major reconstruction is needed (e.g. after a mastectomy or for burnt injuries), the flap is completely separated from the donor site and the vessels are anastomosed to those in the recipient site. In this case, it's fundamental for the flap survival that arteries and veins are well reconnected and no vessels obstruction occurs.

During the operation, imaging techniques are used to check the quality of blood circulation in the arteries and veins. However, the risks of vascular occlusion are important during the first days following the operation. Indeed, the major complication of this type of surgery is thrombosis[1] which can cause the fail of the flap and the consequent necrosis of tissues. In order to save the flap it is fundamental to rapidly restore the vessel permeability: for this reason, the postoperative non-invasive flap perfusion monitoring is critical.

No universally accepted method of flap monitoring exists, and several techniques are in use. Repeated physical examination is most popular but this procedure is highly operator-dependent. Moreover, this examination is even impossible when deep buried flaps are involved (superior than 2 cm in thickness), in case of mastectomy for example where some extra layer of biological tissue is used over the flap to reconstruct the volume of the breast or in case of pharyngeal reconstruction.

Often the physical examination is supplemented with a various instrumental techniques[2] (implantable Doppler system[3], color duplex sonography[4], continuous wave near-infrared spectroscopy[5–7], micro-dialysis[8], laser Doppler flowmetry[9], fluorescent angiography[10,11] etc.). Unfortunately none of them are used routinely because of related disadvantages. For example, Laser-doppler, micro-dialysis or oxygen partial measurement, need to implant a probe in the flap. This implies the need to perforate the flap which exposes to the risk of infection and bleeding or vessel damage. Some of the instrumental techniques are also not able to investigate in depth. This is a severe drawback since in buried flaps (e.g. breast or upper airway reconstruction [12,13]) a fundamental feature is the depth sensitivity. Only the monitoring of perfusion in depth as early as possible can prevent complications.

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In this context, "Time-Resolved Near-Infrared Spectroscopy" (TR-NIRS) has been proven suitable for implementation in portable clinical instruments to measure tissue composition [14]. In particular, in reflectance geometry it represents an attractive technique to non-invasively evaluate the deep perfusion in tissues [15].

Indeed, photons that arrive later are those that travelled most in depth, thus retrieving information about oxygenation of deep layers. This technique is often considered more reliable with respect to continuous wave technique thanks to this information about the photon travelling time, but it also requires expensive, bulky and delicate instrumentation. However, recent results opened the way to a strong improvement in this direction thanks to the use of miniaturized probe-hosted pulsed sources and time-resolved large area detectors, thus knocking-down costs and increasing performances [16,17]. In addition, it has been demonstrated [18,19] that the use of a small distance between injection and collection fibers using a reflectance geometry allows to increase both spatial resolution, the contrast achievable and the overall number of detected photons without any degradation of depth sensitivity, provided that time-gated acquisition are employed to reject the overwhelming number of early photons coming from superficial layers of the tissue under investigation. The use of small distance had already been exploited in different applications like single fiber diffuse optical spectroscopy, non-contact scanning imaging and functional near infrared spectroscopy [20–22].

In [23,24] we presented a setup for Diffuse Optical Tomography (DOT) based on small source-detector distance and high-dynamic range measurements [25] acquired by means of a fast-gated Single-Photon Avalanche Diode (SPAD) [26,27] and analysed using the Mellin-Laplace transform[28] (MLT) and there we demonstrated an increased spatial resolution.

In this work we present a system based on fast wavelengths multiplexing for improving the reconstruction of changes in oxygenation in depth. A pre-clinical study on rats abdominal fascio-cutaneous flaps has been performed so as to prove the suitability of this technique for the non-invasive monitoring of flap perfusion.

2. MATERIAL AND METHODS

1.1. Instrumental setup

A schematic of the setup used is represented in Fig. 1a. Picosecond laser pulses at 40 MHz repetition rate are generated by a supercontinuum fiber laser (Fianium LDT, London, UK). The wavelength was selected by means of three interferential filters centered at 750 nm, 800 nm and 850 nm and mounted on a motorized wheel (software controlled).

We selected those three wavelengths because of their particular position across the oxy- and deoxygenated hemoglobin spectrum (respectively before, on and after the isosbestic point) in order to be able to separately quantify their concentrations. Light exiting from the interferential filter was then attenuated by means of a motorized Variable Optical Attenuator (VOA) and sent into a 1x6 optical fiber switch (core size: 62.5 μm) in order to shine the sample in different positions.

For this proof-of-principle work we shine the sample only in two points of the flap (S1 and S2 in Fig. 1b) and so only two output fibers were selected. Retro-diffused photons were collected in two points (D1 and D2) at the source-detector distance of 10.6 mm. The optical probe consisted into optical fibers fixed in a cylinder with a diameter of 2.5 cm and 16.5 cm long. The probe was kept in place by a mechanical holder. Photons were then focused onto the silicon SPAD (100 μm active area diameter) of the fast-gated module[26].

The laser provided a signal synchronous with optical pulses which was split into four paths: two were sent (passing through a home-made programmable delayer) to fast-gated modules for triggering the gate pulse generation; the other two were used as stop pulses for the two TCPSC boards (SPC 130, Becker&Hickl GmbH, Germany).

The fast-gated acquisitions were fully automatized by means of home-made software. For each wavelength, acquisitions at three different delays from the injection time were taken. Fast-gated SPAD modules collected photons reemitted respectively in D1 and D2, with an integration time of 5 s. The total duration of a complete acquisition was about 3 minutes.

1.2. Flap surgery

Two adult female Wistar rats weighing from 250 to 350 gr were operated. The rats were in individual cages with free and unlimited access to food and water. They were given 15 days of acclimatizing in our institute's animal house before undergoing surgery.

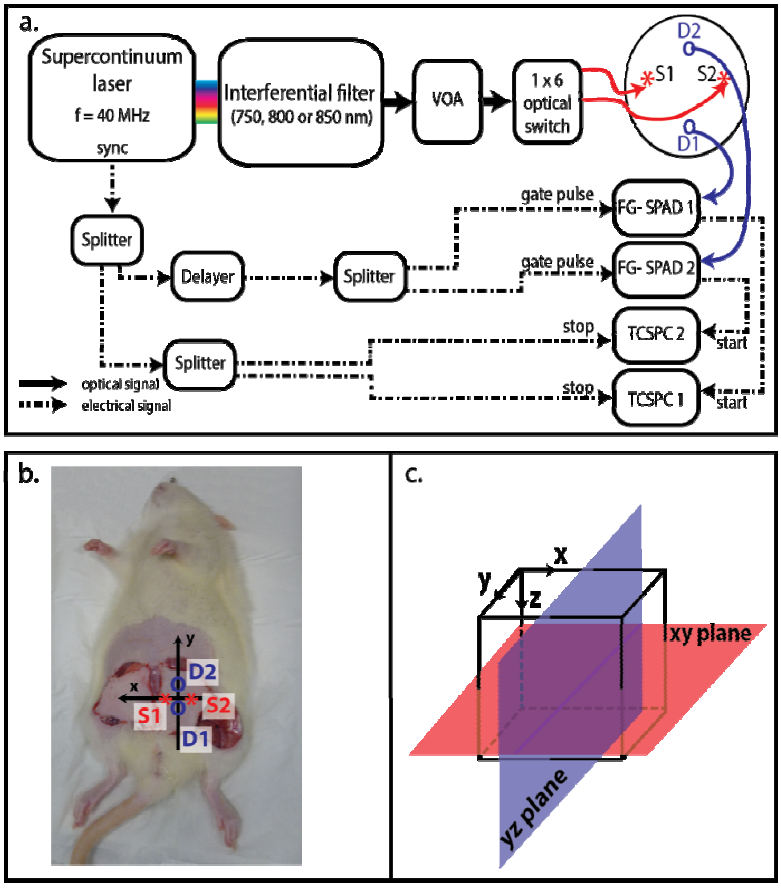

Figure 1. Schematic of the experimental setup (a), position of sources and detectors in the operated mouse (b) and reconstructed volume with axes and representation of xy and yz planes (c).

This research was carried out in accordance with the European convention on the protection of vertebrate animals used for experimental purposes or other scientific purposes (Strasbourg, March 18th, 1986) and in agreement with the rules of the local ethics committee.

After an induction inhalation of isoflurane 4% for 1 minute, the rats had a general anaesthetic by a 1.2 ml intraperitoneal injection with a mixture of ketamine (100 mg/kg) and medetomidine (100µg/kg). A 23 G needle was entirely inserted with a 45° angle in the lower left or right quadrants of the animal's abdomen (on the opposite side to the flap), avoiding the upper quadrants and the median line at the base of the abdomen where the bladder is.

Additional doses (0.2 to 0.3 ml) were administered according to the consciousness level of the animal during the surgical procedure. A test for response to pain is done by heavy pressure on a leg every 30 minutes.

After depilation of the abdomen and the thigh, the skin was prepared with an uncolored antiseptic solution. The rats were installed on their backs on a hot plate at 40° to prevent hypothermia.

The flap harvested half of the skin of the abdominal wall, it measured approximately 35 by 20 mm. It was elevated from the thorax to the thigh controlling the superficial epigastric pedicle on its deep side. With microsurgery tools and 4X magnifier, the pedicle was then dissected and cleared and the vein was separated from the artery. The vein was surrounded by a non-obstructive thread loop. The skin flap was sutured (except in the groin crease) in its initial position (see Fig.1b), leaving the pedicle accessible for later venous clamping.

At the end of experimentation, the rats were euthanized by an intra-cardiac injection of 150 µl Dolethal.

1.3. Data analysis

The measurements were taken with the time-gated technique [29] and they were reconstructed following the procedure described in[23] in order to obtain a large dynamic-range distribution of photon time-of-flight.

The DOT image reconstruction method is described in[28,30] and it was applied to the reconstructed high-dynamic range curves. The direct problem was solved in 3D with the finite volume method on a 4 x 3 x 3 cm mesh grid of regular steps of 0.1 cm in the x, y and z directions (see Fig. 1c) using extrapolated boundary conditions. The "precision" p of the MLT was set to 3 ns^{-1}, and N (number of MLT orders) to 10. Each wavelength (λ) lead to a 3D absorption map $\mu a(\lambda)$ used to compute oxy- and deoxygenated hemoglobin (respectively HHb and HbO_2) concentrations in each pixel of the map.

As a reference state we took the first measure after the occlusion and we described its optical properties by homogeneous maps of $\mu a = 0.2\ cm^{-1}$ and $\mu s' = 10\ cm^{-1}$. Those values were obtained with a previous measurement on a shaved rat. Indeed, the fit of the time-resolved curves with an homogeneous analytical solution of Transfer Equation under the diffusion approximation[31] (with the extrapolated boundary condition[32]) led to a mean optical value of 0.2 cm^{-1} for the absorption and 10 cm^{-1} for the diffusion for the tissue (data not shown here). The solution of the inverse problem was then approached by an iterative method. All the details of the reconstruction method are documented in[28] for theoretical material on the MLT and in[30] for using this algorithm to process experimental signals.

3. RESULTS

Fig. 2 (top) represents maps of HHb and HbO_2 concentrations computed for both yz and xy plane at 3 different times after the starting of the occlusion. Looking at the maps, it is possible to notice that the phenomenon of HHb accumulation is mostly localized between the surface and 5 mm in depth and the maximum increase of HHb is at z = 3 mm. This value is reasonable, considering that the thickness of the flap is about 3-5 mm.

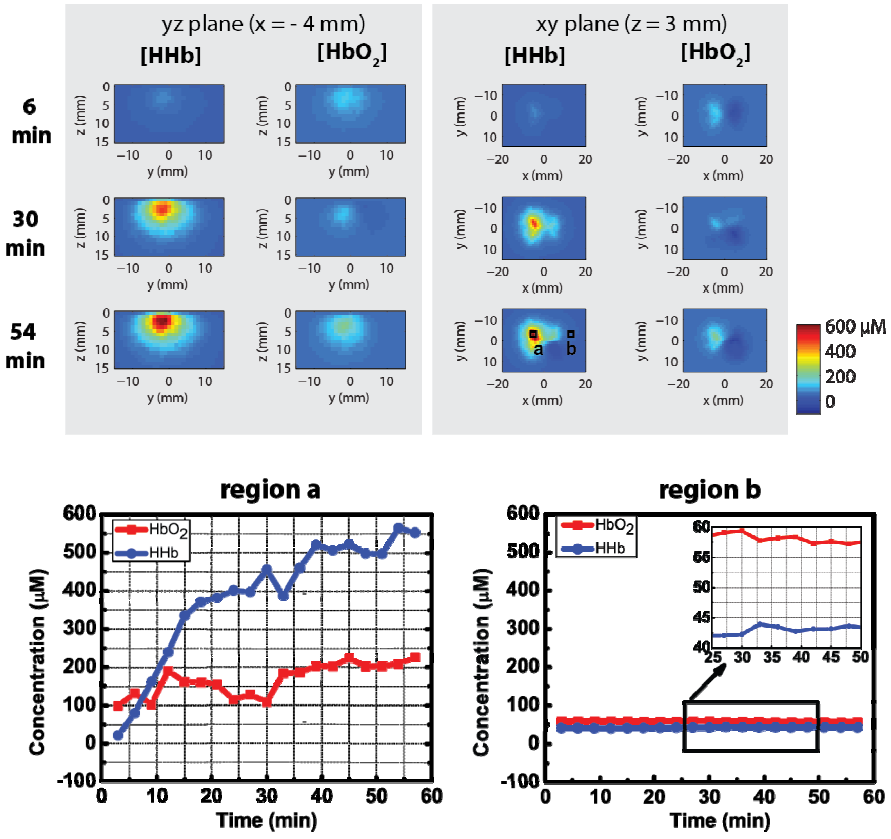

Figure 2. Maps of HHb concentration in the yz plane (x = -4 mm) and xy plane (for z = 3 mm) and time evolution of HHb and HbO_2 concentrations in two different regions (named a and b).

For what concerns time evolution, the accumulation of HHb is clearly visible 30 minutes after clamping of the vein and it increases in time. For HbO_2 no significant variations are present, according to the fact that arteries circulation has not been altered significantly.

On xy plane (plotted at depth of 3 mm) a localized increase in HHb is present in the surroundings of the clamped vein. Moreover, in the HHb and HbO_2 maps in the xy plane, it is possible to notice an area where concentrations of both chromophores are negative. This is due to the initial assumption of a perfectly homogenous reference state, which cannot be the case in reality. This hypothesis should be refined in future work in order to improve the absolute quantification of chromophore concentrations.

In the two graphs on the bottom of Fig. 2 are represented time courses of HHb and HbO_2 computed over a binned area of 2 x 2 pixels. We can notice that in the "region a" a steep increase of HHb occurs in the first 20 min after occlusion beginning and then the value of HHb approaches a constant value. This is most probably due to the formation of a thrombus[33], whose presence had been verified after the end of the experiment. The measured thickness of the thrombus is about 5 mm as we correctly reconstructed in maps where changes in HHb and HbO_2 are limited to 5 mm depth. On the other side, in the "region b" far from the vein no main changes in HHb and HbO_2 occur, as expected. Similar trends have been verified on the other flap performed on the same rat and also on a second rat.

4. CONCLUSIONS

Monitoring flap viability is an unmet clinical need: early diagnosis is needed in order to quickly act in order to save the flap by rapidly restore the vessels permeability. We present a new and fully automatized setup for fast time-gated optical tomography based on two source-detector couples.

We tested the proposed setup for pre-clinical in-vivo measurements on rats, demonstrating its capability to get dynamic 3D tomographic reconstruction of oxy- and deoxygenated hemoglobin. Thanks to the computed maps, we are able to see effects of venous occlusion on oxy- and deoxygenated hemoglobin concentrations.

Indeed, we validated this setup in very preliminary clinical in-vivo measurements on rats where a venous occlusion had been introduced to simulate the fail of reconstructive surgery. From computed maps we can properly detect an increase of HHb and hypothesise the presence of a thrombus.

Thanks to the positive results obtained on rats, we will tackle an animal model closer to the human for flap thickness which is much higher (1-3 cm) than that of rats. In this case the improvement in depth sensitivity due to fast-gated technique will be a fundamental feature. Future works will also consider the optimization of the probe and the increase in source detectors couples in order to improve 3D reconstruction of HHb and HbO_2, reducing artifacts in reconstructed maps and improving spatial resolution.

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