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Broadband (600-1100 nm) Diffuse Optical Characterization of Thyroid Tissue Constituents and Application to *in vivo* Thyroid Studies

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Abstract: We present the first broadband (600-1350 nm) diffuse optical characterization of thyroglobulin and tyrosine, which are thyroid-specific tissue constituents. *In vivo* measurements on the thyroid of 6 subjects enabled their quantification for functional and diagnostic applications.
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1. Introduction

Thyroid plays an important role in the endocrine functions of the human body. Thyroid related pathologies like nodules are a common occurrence, however most of them turn out to be benign with a malignancy rate of 5-10 % [1]. Unfortunately, the palpability of these nodules is less than 5 %, which can reach 19-76 % with ultrasound assistance [2]. A rapid increase in thyroid cancer is observed in the modern society [3]. Importantly, fine needle aspiration (FNA), the established standard to address thyroid pathologies is an invasive biopsy technique with very poor sensitivity , with false negative ranging between 2-37 % [4]. Furthermore, the invasive needle intervention causes pain and discomfort to the patient. These limitations lead to the search of alternative techniques to meet the need for a non-invasive diagnosis of thyroid pathologies.

The preliminary study of thyroid using diffuse optical methods has shown an interesting variation in hemodyamics of healthy and nodular thyroid tissue [5], thus opening further opportunities in the diffuse optical community. However, the study was limited due to short spectral range, and the assumption that oxy and, deoxy-hemoglobin as the only tissue constituents of thyroid which neglected the other tissue constituents that are specific to thyroid organ. Thyroid gland is made of follicles filled with colloid, which is a pool of thyroglobulin protein. Thyroglobulin in turn made of l-tyrosine and iodine, which forms the basic building block of the protein structure [6]. Furthermore, a high concentration of thyroglobulin protein is found in the thyroid, 10-20% in rat thyroid [6]. In conclusion, considering the constituents that are specific to the thyroid organ (especially thyroglobulin and tyrosine) along with typical tissue constituents (lipid, water, collagen, oxy, deoxy- haemoglobin) [7], can lead to new discoveries in the pathological diagnosis of thyroid tissue, as well as in its functional assessment.

In this work, we performed the broadband (600-1100 nm) characterization of these thyroid constituents and explored its non-invasive application to time domain diffuse optical *in vivo* studies at the thyroid region. A portable clinical prototype [8] was used to recover the broadband (600-1100 nm) absorption and reduced scattering spectra of tissue constituents, and to perform *in vivo* measurements on 6 healthy volunteers at 3 locations on the thyroid using 2 source-detector distances (ρ = 1.5 and 2.5 cm) in the wavelength range of 600-1100 nm.

2. Methods

2.1 Instrumentation and analysis

The system is a broadband supercontinuum based TD-DOS spectrometer designed for clinical use [8], and enrolled in various clinical studies [9][10]. The source is a pulsed supercontinuum fibre laser emitting in the range of 450-1750 nm. A Pellin-broca prism is used for spectral tunability which couples the selected wavelength into a 50 µm fibre. Two different detectors namely, a SiPM and an InGaAs photomultiplier tube, are used to effectively cover the whole 600-1350 nm range. The characterisation of tissue constituents (Tyrosine and Thyroglobulin) was performed in

transmittance geometry. The contamination caused by the fluorescence was eliminated using bandpass filters of 10 nm bandwidth [7].
The curve fitting was performed on acquired temporal curves using the solution of the Diffusion equation with the extrapolated boundary conditions for a homogeneous medium convoluted with the instrument response function. For *in vivo* measurements, following a primary fit using diffusion equation, a secondary fit was performed to quantify tissue constituents.

2.2 In vivo study

Measurements were performed on 6 healthy subjects at three locations around thyroid region. Prior to the study, each subject signed an informed consent form. Diffuse optical measurements were performed in reflectance geometry at two different source-detector separations (d = 1.5, 2.5 cm). Assistance of ultra-sound imaging was exploited to locate the thyroid organ. Measurements were acquired for 1 second at each wavelength in reflectance geometry over 600-1100 nm with 10 nm as step size.

3. Results and Discussion

Fig. 1 shows the results of tissue constituents' characterisation, both thyroglobulin and tyrosine have distinguishable spectral features that can enable their quantification with minimum coupling between each other. A smooth spectrum was observed for thyroglobulin (Fig. 1 (a)) with a shoulder around 1000 nm followed by major peak at 1190 nm. In case of tyrosine (Fig. 1 (b)), 6 peaks were seen at different parts (875 nm, 915 nm,1090 nm, 1145 nm, 1185 nm, 1290 nm) of the broadband window (data not shown). The absorption of tyrosine reaches close to zero around 800 nm. In-spite of low density, thyroglobulin has high absorption as compared to tyrosine, which increases its detectability at low concentrations.

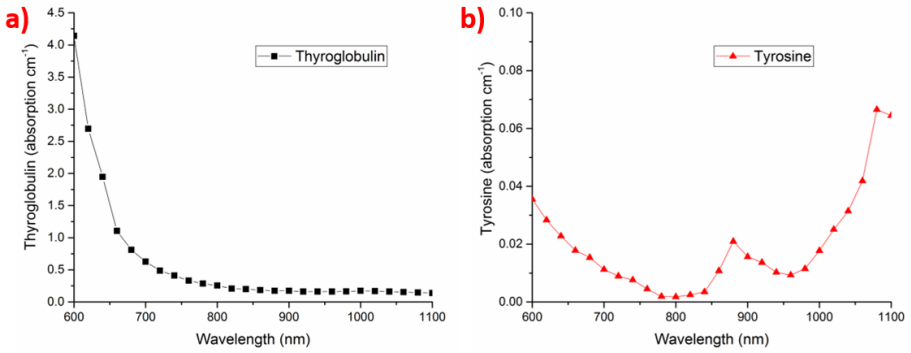

Fig. 1. Absorption and reduced scattering spectra of (a) thyroglobulin, (b) tyrosine.

Fig. 2 (a)(b), shows the mean optical properties of thyroid at two different source detector separations (d = 1.5 cm, 2.5 cm) and of tissue at a reference point (external to the thyroid area) (Fig 2(c): position 3). The mean and standard deviation for measurements at different thyroid locations (positions 1,2 in Fig 2(c)) are shown in Fig. 2 (a)(b) for the two values of d. The absorption spectrum at the reference position (violet squares) appears different from that of the thyroid locations, with significantly lower absorption at all wavelengths. However, the measurements at two different source-detector separations are found to be similar, with measurements at short d (1.5 cm) showing slightly higher absorption around 700 and 1070 nm. This suggests that the measurements at d = 1.5 cm may reflect higher collagen content.

Interestingly, before performing the present study, the secondary fit performed in the absence of the new tissue constituents always overestimated (40-60%) the concentrations of lipid, water, and collagen. This led us to the hypothesis of missing components. The results of tissue constituent quantification are shown in Fig. 3 for major hemodynamic parameters (Hb, HbO_2, THb, SO_2) and tissue constituents (lipid, water, collagen), together with the new thyroid-specific constituents (thyroglobulin (TG), tyrosine (T)). From Fig. 3, both source-detector separations lead to similar tissue constituent values. The high collagen value at for short (d = 1.5 cm) is result of higher absorption at long and short wavelengths. At short d, the light explores shallower regions of the diffusive medium as compared to large source-detector separation (d = 2.5 cm), and a comparatively high collagen content at 1.5 cm is likely due to higher content in superficial tissues as compared to the deeper thyroid organ. Tyrosine and thyroglobulin were quantified based on the spectra presented in Fig. 1. This quantification proves our hypothesis of missing components, when they are neglected.

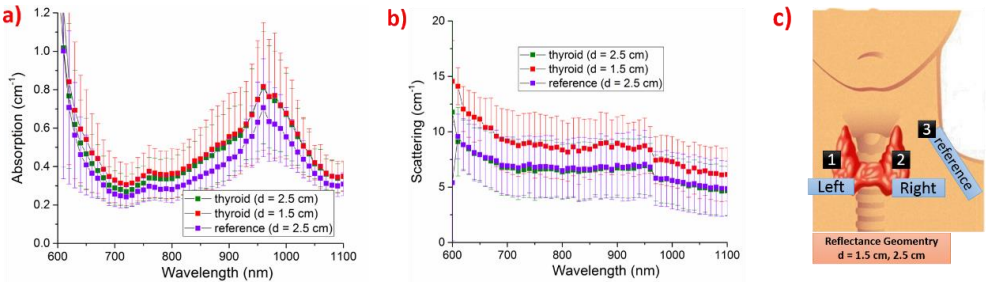

Fig. 2. Absorption (a) and scattering (b) properties of thyroid at two source detector separations (red squares (d = 1.5 cm), green squares (d = 2.5 cm)), and of the reference location (violet square). (c) Schematic diagram of protocol locations.

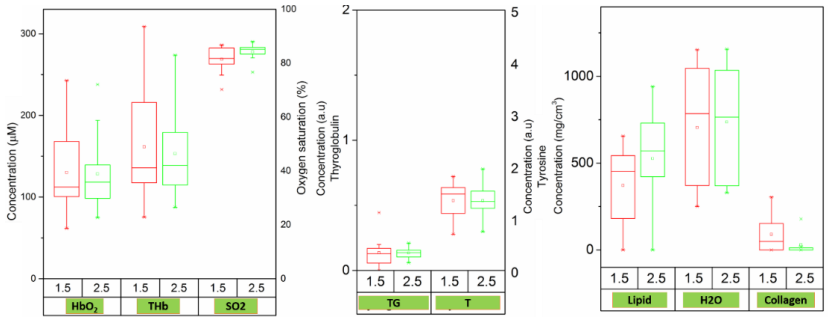

Fig. 3. Box plots of hemodynamic parameters (Hb, HbO_2, THb, SO_2), major tissue constituents (lipid, H_2O, collagen), and constituents (thyroglobulin (TG), tyrosine (T)) that are specific to thyroid.

4. Conclusions

We have presented a first optical characterization of thyroglobulin and tyrosine which are tissue constituents specific to thyroid organ using the broadband (600-1100 nm) TD-DOS technique, identifying distinctive spectral features. Furthermore, we have applied TD-DOS to the non-invasive *in vivo* characterization on the thyroid region, a significantly higher absorption was found for thyroid region as compared to the surrounding tissue. Analysis of the absorption spectra with linear combination of tissue constituents' spectra allowed the first *in vivo* quantification of thyroid constituents, and in particular of its tyrosine and thyroglobulin content.

5. Acknowledgements

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