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Thyroid Tissue Constituents Characterization and Application to *in vivo* studies by Broadband (600-1200 nm) Diffuse Optical Spectroscopy

Sanathana Konugolu Venkata Sekar[1], Andrea Farina[2], Alberto dalla Mora[1], Paola Taroni[1,2], Claus Lindner[3], Mireia Mora[4], Parisa Farzam[3], Marco Pagliazzi[3], Mattia Squarcia[4], Irene Halperin[4], Felicia A. Hanzu[4], Hamid Dehghani[5], Turgut Durduran[3,6], Antonio Pifferi[1,2]

[1]*Dipartimento di Fisica, Politecnico di Milano, Milano, Italy*
[2]*Consiglio Nazionale delle Ricerche, Istituto di Fotonica e Nanotecnologie, Milano, Italy;*
[3]*ICFO-Institut de Ciències Fotòniques, The Barcelona Institute of Science and Technology, 08860 Castelldefels (Barcelona), Spain*
[4]*IDIBAPS, Fundació Clínic per la Recerca Biomèdica, 08036 Barcelona, Spain*
[5]*School of Computer Science, University of Birmingham, Edgbaston (Birmingham), United Kingdom*
[6]*Institució Catalana de Recerca i Estudis Avançats (ICREA), 08015, Barcelona, Spain*
sanathana.konugolu@polimi.it

Abstract:
We present the first broadband (600-1100 nm) diffuse optical characterization of thyroglobulin and tyrosine, which are thyroid-specific tissue constituents. *In-vivo* measurements at the thyroid region enabled their quantification for functional and diagnostic applications.
OCIS codes: (170.5280) Photon migration; (170.6510) Spectroscopy, tissue diagnostics; (170.6935) Tissue characterization; (170.3890) Medical optics instrumentation

1. Introduction

Thyroid hormone secretion plays a crucial role in the endocrine functions of the human body. Thyroid nodules are a common occurrence, but most of them turn out to be benign with a malignancy rate of 5-10 % [1]. Recent studies have revealed that the incidence of thyroid cancer is rapidly increasing in the modern society [2]. Unfortunately, the palpability of these nodules is less than 5 %, and with ultrasound assistance can reach 19-76 % [3]. Importantly, fine needle aspiration (FNA), the established standard to address thyroid pathologies like thyroid cancer through cytological analysis, is an invasive biopsy technique. Furthermore, the sensitivity of FNA is quite low, with false negative ranging between 2-37 % [4] and also an invasive needle intervention causes pain and discomfort to the patient. These limitations lead to the search for alternative techniques to meet the need for a non-invasive diagnosis of thyroid pathologies.

The diffuse optical study of thyroid is still in its nascent stage, work of Linder et al.[5], showed interesting variations in hemodynamics of healthy and nodular thyroid tissue, thus opening further interests in the diffuse optical community. However, this study involved a limited spectral range, and considered only oxy and deoxy-hemoglobin as tissue constituents, neglecting the other tissue constituents that are specific to thyroid organ. Thyroid is a brownish-red gland with follicles filled with colloid, which is a pool of thyroglobulin protein. Thyroglobulin in turn consists of l-tyrosine and iodine, which are the basic building blocks of the thyroglobulin protein structure [6]. Importantly, thyroglobulin protein concentration in thyroid is relatively high: studies on the rat thyroid reveal it to be around 10-20 % [6]. In conclusion, along with typical tissue components (lipid, water, collagen, oxy, deoxy- haemoglobin), considering the constituents that are specific to the thyroid organ (especially thyroglobulin and tyrosine) can lead to new discoveries in the pathological diagnosis of thyroid tissue, as well as in its functional assessment.

To the best of our knowledge, in literature there is the lack of thyroglobulin and tyrosine absorption spectra in the visible-NIR region. Unlike transparent samples, the highly diffusing nature and fluorescence of these substances in the visible range make their characterization impossible with a traditional spectrophotometer. Diffuse optical techniques can be an optimal option. Especially, time domain diffuse optical spectroscopy (TD-DOS), with the advantage that it naturally disentangles absorption from scattering, can characterize effectively the optical properties of diffusing media like human bone [7][8], tissue phantoms [9] etc.

In this work, we performed the characterization of these thyroid constituents and explored its application to time-domain diffuse optical *in vivo* studies at the thyroid region. To this end, a portable clinical prototype for TD-DOS [10][11] 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 4 locations on the thyroid using 2 source-detector distances (ρ = 1.5 and 2.5 cm) in the wavelength range of 600-1200 nm.

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2. Methods

2.1 Instrumentation and analysis

The instrumentation is a broadband TD-DOS spectrometer designed for clinical use [10]. The source is a pulsed supercontinuum fibre laser emitting in the 450-1750 nm range. Spectral tunability is achieved using a Pellin-Broca prism and coupling the selected wavelength into an optical fibre. Two different detectors, namely a SiPM [12] and an InGaAs PMT, are used to cover the whole 600-1350 range. Tissue constituent characterisation was performed in transmittance geometry. Bandpass filters of 10 nm were used to eliminate fluorescence contamination.
The acquired temporal curves were fitted using the solution of the Diffusion equation under 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

In total, 6 subjects were enrolled in the protocol. Informed consent was signed by all subjects prior to the study. Diffuse optical measurements were performed in reflectance geometry at two different source-detector separations (d = 1.5, 2.5 cm). Four locations, as shown in Fig. 2(c), were chosen for thyroid studies. Assistance of ultra-sound imaging was exploited to locate the thyroid organ. Measurements were performed over 600-1200 nm with 10 nm as step size for an acquisition period of 1 second at each wavelength.

3. Results and Discussion

The results of tissue constituents' characterisation are shown in Fig. 1. All three constituents have distinguishable spectral features that can enable their quantification with minimum coupling between each other. Thyroglobulin is found to have smooth decreasing spectrum with a shoulder around 1000 nm. Tyrosine has 2 peaks at different locations (875 nm and 1090 nm) of the broadband (600-1100 nm) window. Moreover, its absorption reaches close to zero around 800 nm and a shoulder is observed around 915 nm. Thyroglobulin has high absorption as compared to tyrosine which makes it easier to detect at low concentrations.

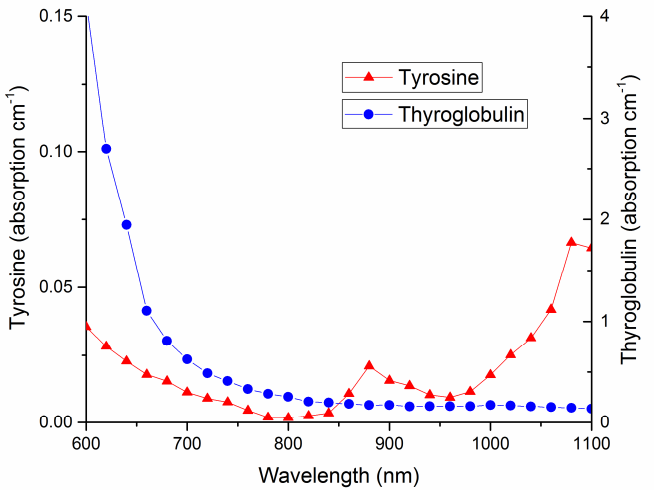

Fig. 1. Absorption and reduced scattering spectra of tyrosine (right axis), thyroglobulin (left axis).

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 at the reference point (Fig 2(c): position 4). The mean and standard deviation for measurements at different thyroid locations (Fig 2(c): position 1,3) are shown in Fig. 2(a-b) for the two values of d. The absorption spectrum at the reference position (violet circles) seems to be highly different from that of the thyroid locations, with absorption values significantly lower 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, 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. Along with typical major hemodynamic parameters (deoxyhaemoglobin Hb, oxyhaemoglobin HbO_2, total haemoglobin content THb, oxygen saturation level SO_2) and tissue constituents (lipid, water, collagen), we considered the new tissue constituents (thyroglobulin (TG), tyrosine (T)) that are specific to thyroid organ. From Fig. 3, both source-detector separations were found to have similar tissue constituent values. The higher absorption at both short and long wavelengths for small source-detector separation (d = 1.5 cm) thyroid measurements resulted in higher collagen value. 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 deeper thyroid organ. Tyrosine (T) and thyroglobulin (TG) were quantified based on the spectra presented in Fig. 2. This quantification proves our hypothesis of missing components, when they are neglected.

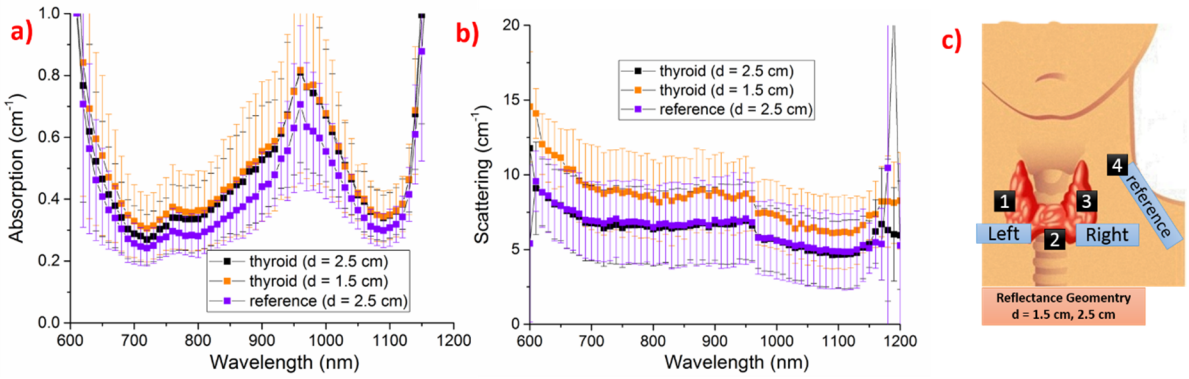

Fig. 2. Absorption (a) and scattering (b) properties of thyroid at two source detector separations (red triangles (d = 1.5 cm), black squares (d = 2.5 cm)), and of the reference location (blue circles). (c) Schematic diagram of protocol locations.

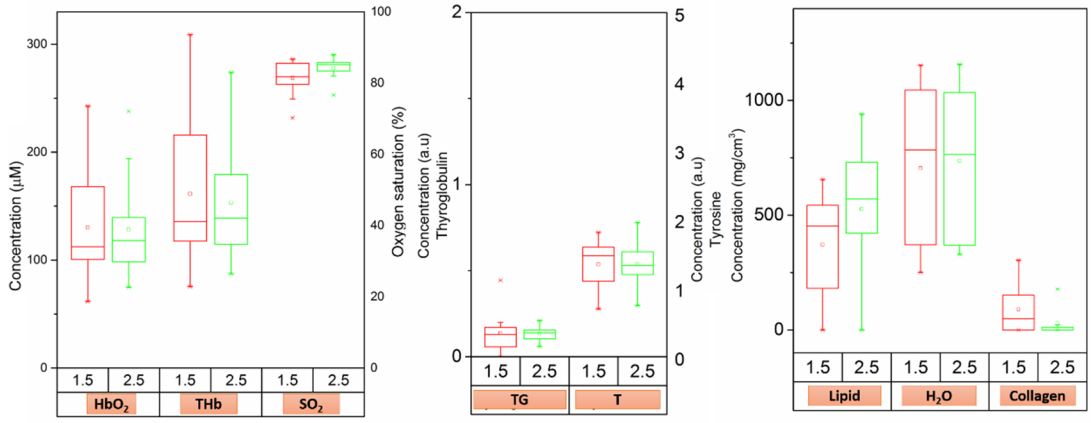

Fig. 3. Box plots of hemodynamic parameters (Hb, HbO_2, THb, SO2), major tissue constituents (lipid, water, collagen), and constituents (thyroglobulin (TG), tyrosine (T)) that are specific to thyroid.

4. Conclusions

We have presented a first optical characterization of tyrosine and thyroglobulin using the broadband TD-DOS technique, identifying distinctive spectral features in these thyroid specific constituents. Further, we have applied TD-DOS to the non-invasive *in vivo* characterization on the thyroid region, finding significantly higher absorption over the thyroid as

compared to the surrounding tissue. Analysis of the absorption spectra with a combination of the main tissue absorbers permitted a first *in vivo* quantification of thyroid constituents, and in particular of its tyrosine and thyroglobulin content.

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