

# Spectroscopic Analysis of Parathyroid And Thyroid Tissues By Ground-State Diffuse Reflectance and Laser Induced Luminescence : A Preliminary Report

Carlos Serra (✉ [caaserra@me.com](mailto:caaserra@me.com))

Hospital SAMS <https://orcid.org/0000-0003-3320-3464>

João Serra

INESC Microsystems and Nanotechnologies: INESC Microsistemas e Nanotecnologias

Isabel L Ferreira-Machado

Polytechnic Institute of Portalegre: Instituto Politecnico de Portalegre

Luís F Vieira-Ferreira

Universidade de Lisboa Instituto Superior Técnico: Universidade de Lisboa Instituto Superior Tecnico

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## Short Report

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# Abstract

Intraoperative discrimination of thyroid and parathyroid tissues is fundamental in thyroid surgery. Recent fluorescence studies have shown stronger NIR emission in parathyroid tissue than in thyroid tissue, presenting a potential avenue for the development of a tool for surgical assistance. However, the fluorophore responsible for this emission has not yet been identified. In this work, spectroscopic analysis was performed to ascertain the origin of the emission peaks in parathyroid tissue. Ground-state diffuse reflectance (GSDR) absorption spectroscopy and laser-induced luminescence (LIL) emission spectroscopy were performed in parathyroid, thyroid, and fatty tissue samples and the resulting spectra were compared with the peaks of known fluorophores to identify the origin of each peak. The spectra of the different tissue types were also compared in order to evaluate the wavelength which presents the highest parathyroid emission relative to the emission of the surrounding tissues, representing the ideal wavelength for parathyroid detection. An emission peak in these conditions was observed for both thyroid and parathyroid tissue at 711 nm, with a higher intensity in parathyroid sample, making it suitable for detection applications. These results show a potential avenue for the development of a system allowing parathyroid detection in a surgical setting.

## Introduction

Intraoperative identification of parathyroid glands during thyroid or parathyroid surgery is always a complex task for surgeons, as the small size, color and shape of these glands makes difficult their discrimination from lymph nodes and cervical fat, and highly dependent on the surgeon's experience [1].

In 2011 investigators from Vanderbilt University [2] described autofluorescence of parathyroid glands with maximum emission at 822 nm when submitted to a 795 nm laser beam. Even though thyroid gland also emits fluorescence at the same wavelength, its lower intensity allows their discrimination from parathyroid. That revelation was received with expectations by endocrine surgeons since it could be used for the development of devices for intraoperative utilization. Other authors confirmed the initial findings of Paras et al, and several devices were described [1, 3–7].

The origin of parathyroid glands near infrared autofluorescence at 822 nm is still unknown. No natural fluorophores are known at that wavelength. The responsible fluorophore must exist in thyroid and parathyroid, with higher concentration in parathyroid and be absent in other cervical tissues. Some possible responsible fluorophores that fit the conditions, such as the Calcium Sensing receptors and the Vitamin D receptors, were discarded by previous works [8].

In this work, thyroid and parathyroid tissues were analyzed by ground-state diffuse reflectance (GSDR) absorption spectroscopy and laser-induced luminescence (LIL) emission spectroscopy, in order to obtain experimental information leading to the responsible fluorophore. The absorption and emission spectra of parathyroid and thyroid tissue were also compared to identify other wavelengths that can allow better discrimination between tissues.

# Materials And Methods

Thyroid, parathyroid and fat tissue samples were acquired from clinical patients during surgeries for multinodular goiter (thyroid) and parathyroid adenoma (parathyroid and fat). Tissues were conserved in formaldehyde to prevent tissue degradation.

GSDR and LIL spectra were acquired at the Surface Photochemistry Laboratory, from the Surface Photochemistry Group from BSIRG, at Instituto Superior Técnico. GSDR was performed using a homemade setup containing a 250W W-Hal lamp as monitoring lamps. A fixed monochromator (Oriel, FICS 77441) coupled to an ICCD detector (Andor, i-Star 720) with time gate capabilities was used in the accumulation mode, was used to detect the reflectance signals. The signals were collected by a collimating beam probe coupled to an optical fibre (fused silica) in this way assuring the connection to the monochromator entrance. The reflectance  $R$  is obtained for each sample and the remission function is calculated with the equation  $F(R) = (1 - R)^2/2R$ . Further details of the use of this absorption spectroscopic technique can be found in [9–12] The absorption spectra of parathyroid, thyroid and fatty tissue from human subjects have been acquired in the range between 200 nm and 800 nm with the use of this technique.

LIL studies were performed using again the i-Star 720 ICCD (with a minimum temporal gate of 2.2 ns), with 1024 x 128 pixels in a maximum spectral range of 200 to about 900 nm and also with a Andor i-Dus in the near infra-red (NIR) spectral range (700 to 1700 nm). Cut-off filters at 695 nm and 850 nm were used to attenuate the signal from the excitation wavelength. Laser sources with wavelengths of 650 nm and 785 nm were used to excite the sample. The acquisition setups are fully described in [9–12].

## Results

Figure 1 shows the absorption spectra of 3 parathyroid samples acquired using GSDR between 200 nm and 800 nm. The main absorption peaks are visible at 220 nm, 275 nm, 411 nm, 545 nm and 580 nm. All three samples show peaks in the same wavelengths, with the only significant difference being the relative amplitude of each peak between different samples. Spectra of thyroid, and fatty tissue samples have also been acquired between 200 nm and 800 nm (Fig. 2).

Clearly, the absorption spectrum of the 3 parathyroid samples also evidence absorption of the fatty tissue (maximum absorption at 411 nm). The main point to stress is the absorption tail that exists in the 600 nm to 850 nm spectral range which is clearly absent in the thyroid and fatty tissue samples, where a cut-off of exists at about 600 nm. This important absorption that reaches the NIR spectral range is assigned by us as a characteristic absorption of the parathyroid tissue.

The emission spectra of thyroid and parathyroid tissue were also acquired with an excitation wavelength of 650 nm and a cut-off filter at 695 nm, in order to identify other fluorescent peaks that can provide a better contrast between the two types of tissue. By choosing an excitation of 650 nm, located after the cut-off of the thyroid or fatty tissue, we excite predominantly the parathyroid tissue, aiming to observe the

specific emission spectra of this tissue. All these spectra are represented in Fig. 3. Both thyroid and parathyroid spectra show a single peak at 711 nm. Taking into account the average of the 3 thyroid acquisitions and the 3 parathyroid acquisitions, the peak intensity was found to be  $2.77 \pm 0.21$  times higher in the parathyroid samples than in thyroid samples. This significantly higher emission in parathyroid tissue can be potentially used in a surgical setting for tissue discrimination.

The NIR emission of the parathyroid sample was also acquired using the i-Dus detector, now with an excitation wavelength of 785 nm and a cut-off filter of 850 nm (spectrum presented in Fig. 4). A long tail in the NIR spectral region could now be observed, due to the high sensitivity of this detector in the NIR range. This tail is obviously not detected with the use of the i-Star ICCD, because this one only operates until about 850 nm.

## Discussion

Parathyroid shows absorption peaks at 220 nm, 275 nm, 411 nm, 545 nm and 580 nm (Fig. 1). In literature, peaks at 545 nm and 580 nm have only been observed in malignant tissue [13].

The absorption peak at 275 nm has been theorized to originate due to the presence of collagen, elastin and tryptophan [14, 15].

The absorption band between 400 nm and 600 nm, present in all tissue types, has been attributed to the presence of hemoglobin in the analysed tissue [16], with the most intense peak at 411 nm also being in the emission range of several chromophores, such as protoporphyrin and FAD (flavin adenine dinucleotide) [13], which are present in all types of analysed tissue.

The NIR emission of parathyroid tissue (Fig. 4) with excitation at 785 nm is dominated by a peak at 910 nm, which is simply due to the used cut-off filter. References to a very intense peak at around 825 nm are found in literature [1–3], which also may be accounted for the acquired peak after attenuation by the cut-off filters under use. The emission peak observed by us at 711 nm is present in both thyroid and parathyroid tissue. However, a significant higher intensity was observed in the case of the parathyroid tissue, hinting that a single chromophore present in both types of tissue with a higher concentration in parathyroid tissue is responsible for this peak [1], as Fig. 3 clearly shows.

With excitation at 650 nm (Fig. 3), a single peak is observed in the emission spectra of both thyroid and parathyroid tissue (at 711 nm), which has previously been attributed to the presence of protoporphyrin [15]. The significantly higher intensity of this peak in the parathyroid tissue presents a potential avenue for a future technique for optical distinction between the different types of tissue in surgery.

## Conclusions

The origin of the NIR emission of parathyroid tissue reported in literature has not been identified fully, although experimental data point to a protoporphyrin luminescence. The experiments performed using

both LIL and GSDR in the considered ranges have not been sufficient to fully determine the chromophore responsible for increased emission in parathyroid tissue when compared to thyroid samples. The presence of collagen, elastin and tryptophan in higher concentrations in parathyroid tissue may also be an evidence of the contribution of these chromophores to the higher emission intensity in parathyroid.

An emission peak at 711 nm was obtained when using an excitation wavelength of 650 nm, with a stronger intensity for parathyroid tissue than thyroid tissue. These results provide an alternative wavelength to be used for surgical guidance with excitation in the visible range. If adapted to a surgical setting, this would allow the surgeon to visualize and aim the excitation light source during surgery with only the operator's vision, with complex imaging equipment being required only for the visualization of the induced fluorescence and not to visualize the excitation beam.

## **Declarations**

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### **Funding**

No funding was received for this work

### **Conflicts of interest/Competing interests**

Nothing to declare

### **Ethics approval**

Collection of samples was authorized by Ethics Comittee of Hospital dos SAMS (approval 002/2018)

### **Consent to participate**

Not applicable

### **Consent for publication**

Not applicable

### **Availability of Data and Matherials**

Data available on request

## Code Availability

Not applicable

## Authors Contributions

Study conception and design: CS, JS , IFM, LVF

Acquisition of data: CS, JS , IFM, LVF.

Analysis and interpretation of data: CS, JS , IFM, LVF

Drafting of the manuscript: CS, JS.

Critical revision of the manuscript: IFM, LVF

All authors read and approved this manuscript

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## Figures

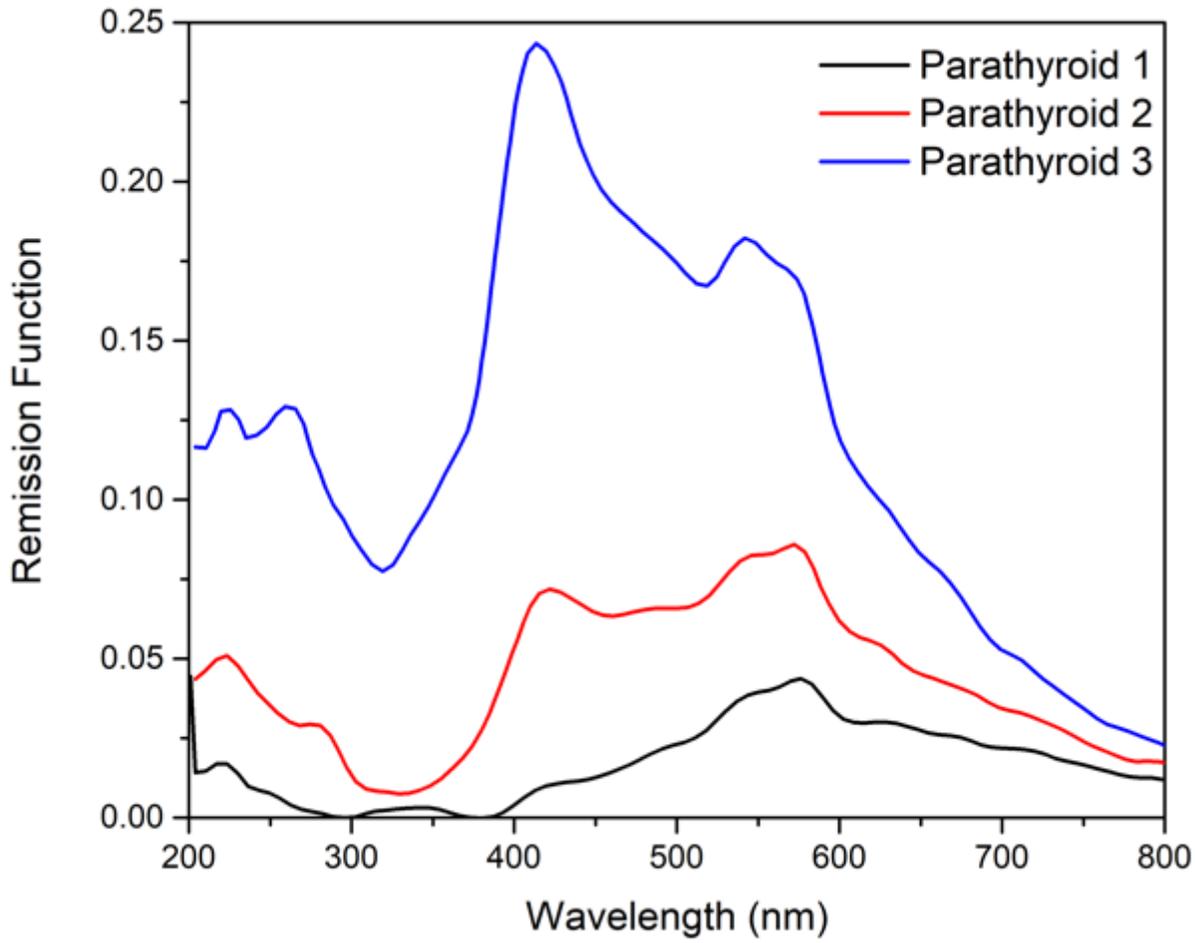


Figure 1

Absorption spectrum of parathyroid samples acquired using GSDR.

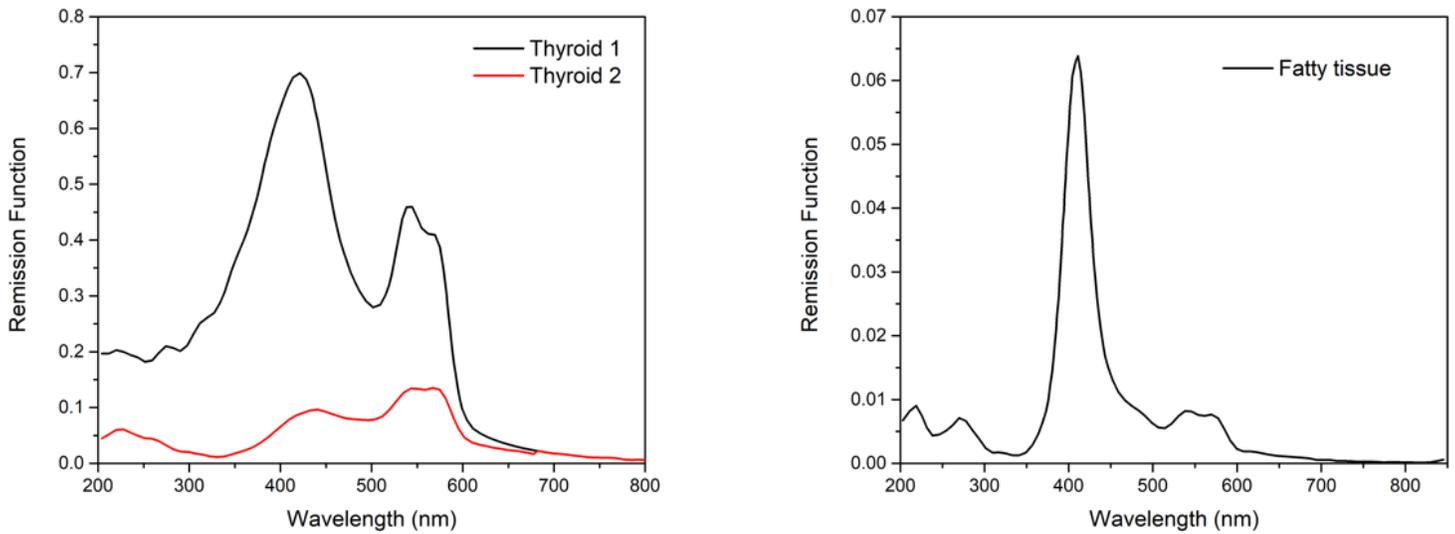
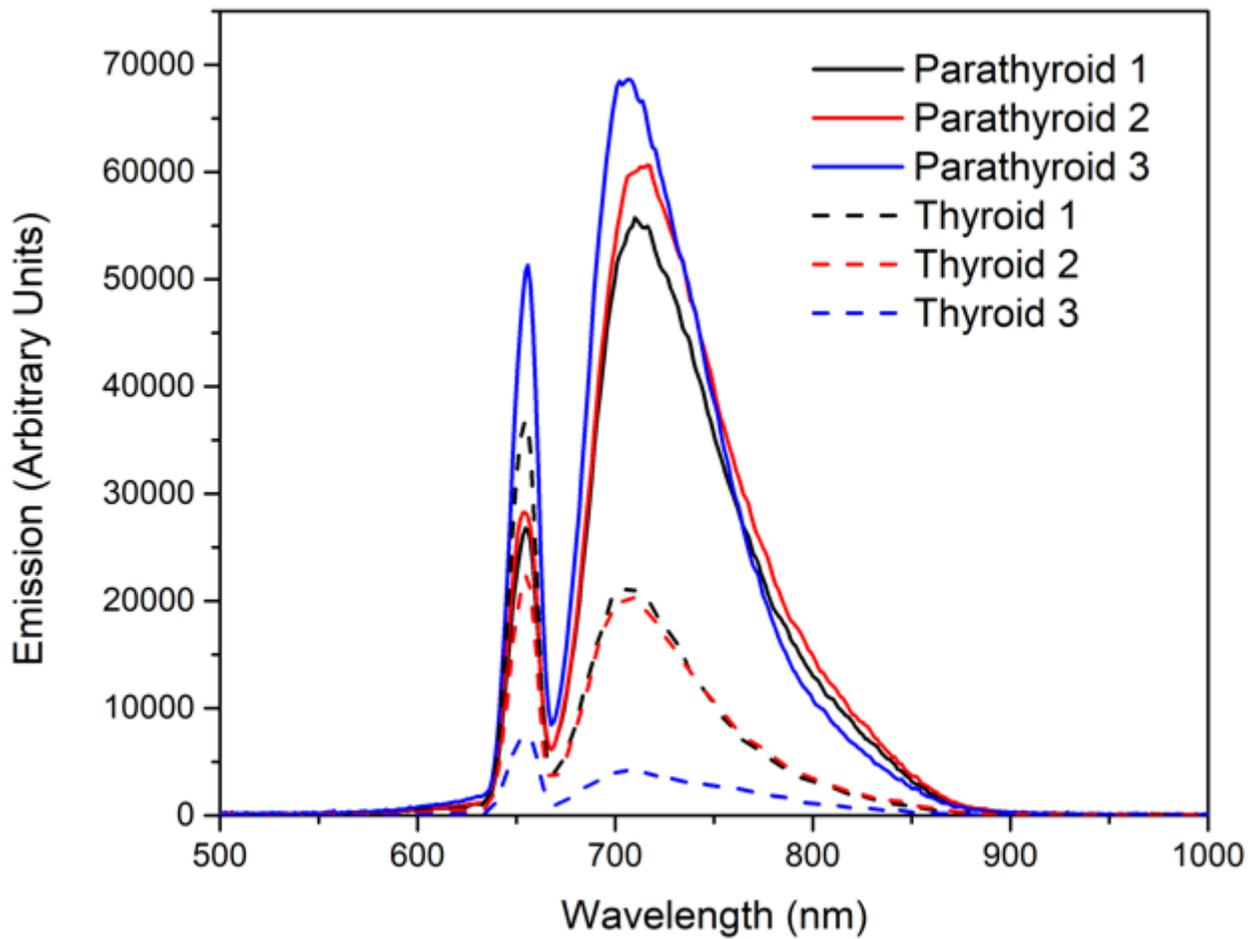


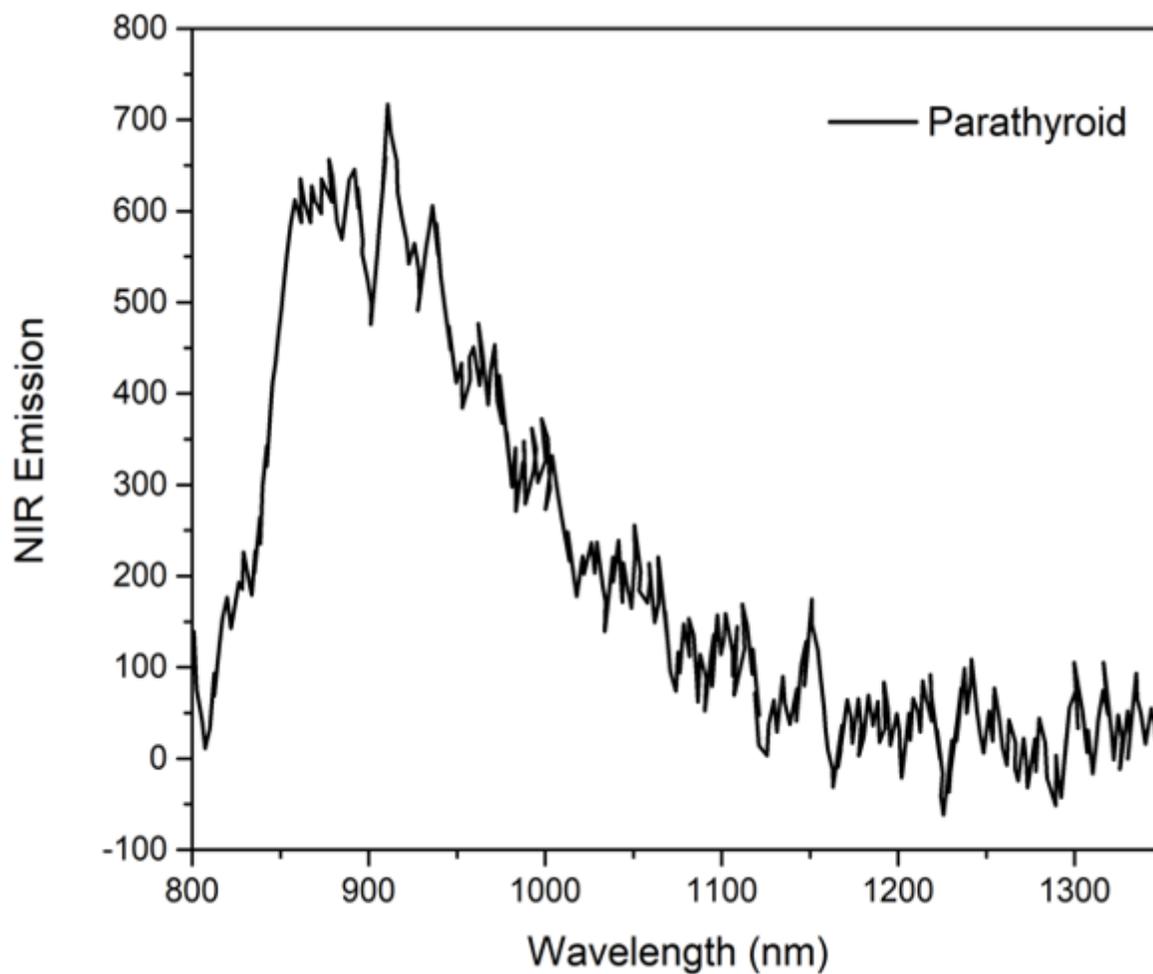
Figure 2

Absorption spectra of different tissues acquired using GSDR. (a) Thyroid; (b) Fatty tissue.



**Figure 3**

Emission spectra of thyroid and parathyroid samples with excitation at 650 nm. The peak at 650 nm corresponds to the incident laser, which was not fully attenuated by the cut-off filter at 695 nm.



**Figure 4**

NIR emission spectrum of parathyroid samples acquired by LIL. Excitation at 785 nm with a cut-off filter at 850 nm.