

Journal of Biomedical Optics

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Abstract. A major challenge in endocrine surgery is the intraoperative detection of parathyroid glands during both thyroidectomies and parathyroidectomies. Current localization techniques such as ultrasound and sestamibi scan are mostly preoperative and rely on an abnormal parathyroid for its detection. In this paper, we present near-infrared (NIR) autofluorescence as a noninvasive, real-time, automated *in vivo* method for the detection of the parathyroid gland. A pilot *in vivo* study was conducted to assess the ability of NIR fluorescence to identify parathyroid glands during thyroid and parathyroidectomies. Fluorescence measurements at 785 nm excitation were obtained intra-operatively from the different tissues exposed in the neck region in 21 patients undergoing endocrine surgery. The fluorescence intensity of the parathyroid gland was found to be consistently greater than that of the thyroid and all other tissues in the neck of all patients. In particular, parathyroid fluorescence was two to eleven times higher than that of the thyroid tissues with peak fluorescence occurring at 820 to 830 nm. These results indicate that NIR fluorescence has the potential to be an excellent optical tool to locate parathyroid tissue during surgery. © 2011 Society of Photo-Optical Instrumentation Engineers (SPIE). [DOI: 10.1117/1.3583571]

Keywords: biomedical optics; fluorescence; fluorescence spectroscopy; biophotonics.

Paper 10498LR received Sep. 23, 2010; revised manuscript received Apr. 4, 2011; accepted for publication Apr. 7, 2011; published online Jun. 8, 2011.

Disease of the parathyroid and thyroid glands are common, with 25 to 28 cases per 100,000 of hyperparathyroidism and approximately 20 million people affected by some sort of thyroid disease.^{1,2} Surgical means are often the subsequent recourse used to treat the affected gland(s).³ Endocrine surgery has traditionally involved meticulous dissection and resection of diseased glands while leaving the normal glands intact. Serious complications can occur when one or more of the parathyroid glands are incompletely removed during parathyroidectomies or unintentionally injured or removed during thyroidectomies.⁴ The incidence of inadvertent parathyroidectomy ranges from 8 to 19% in patients undergoing total thyroidectomy and is dependent on the experience of the surgeon.⁵ Such accidental removal or injury of the parathyroid may lead to complications such as postoperative hypocalcaemia and hypoparathyroidism that may have lifelong deleterious consequences on calcium homeostasis.

The parathyroid glands can be difficult to visually distinguish during surgery because of their small size and appearance that is often similar to lymph nodes, fat, and occasionally thyroid tissue. In addition, parathyroid identification is often confounded by variability in the location of the glands and overlying layers of fat. Existing methods rely on histopathology or post-operative evaluation to determine if the parathyroids were accidentally or incompletely removed.⁶ Surgical biopsy of the parathyroid for identification can lead to devascularization and

destruction of the functional gland; consequently, surgeons must ultimately rely on visual inspection to identify the different tissues, which can be subjective and inconclusive, especially for an inexperienced surgeon.^{7,8} In fact, thyroidectomies are typically performed by general or ear, nose, and throat surgeons, rather than endocrine surgeons, thus, an accurate automated tissue identification method would allow safer more effective patient management.^{9,10}

The goal of this study then was to develop an optical method to intra-operatively discriminate parathyroid tissue from all other anatomical structures in the neck. This paper presents a method for identification of parathyroid tissue regardless of disease state based on intrinsic near-infrared (NIR) autofluorescence. In a pilot study, data was collected *in vivo* from 21 patients undergoing surgery. In every patient, parathyroid tissue exhibited more intense autofluorescence above 800 nm allowing us to distinguish it from the surrounding tissue.

Measurements were performed at the Vanderbilt University Medical Center under approval by the Vanderbilt Institutional Review Board. All patients with primary thyroid or parathyroid pathophysiology undergoing thyroid/parathyroidectomy were considered. Initial evaluation was conducted by the participating endocrine surgeon (Dr. John Phay) while seeing the patients at the Vanderbilt Clinic and final eligibility was preoperatively determined based on the clinical condition and safety of the patient. Twenty-one patients, aged 18 to 99, regardless of race and gender, were enrolled in the study following informed written consent.

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Near-infrared fluorescence was excited with a 785-nm diode laser (U-type, IPS, Monmouth Junction, New Jersey) that delivered 80 mW at the tissue surface with a spot size of 400 μm . Fluorescence spectra were detected using a fiber optic spectrometer (S2000-FL, Ocean Optics, Dunedin, Florida) with a spectral resolution of 10.5 nm (FWHM). The entire system is computer controlled by custom software developed in Lab-View (National Instruments, Austin, Texas). Light was delivered and collected from the tissue site with a 6-around-1 sterilized fiber optic probe. Inline filtering in the probe prevents 785 nm light from interfering with the collected fluorescence light.¹¹ An additional 3-mm diameter longpass filter was placed in the fiber port of the spectrometer to further reduce the amount of 785 nm light entering the detector.

Fluorescence spectra were measured from multiple locations in the thyroid, parathyroid, fat, muscle, and lymph depending on the accessibility of the tissues. All measurements were made with the room and operating lights turned off. The fiber optic probe was firmly placed in contact during each measurement while maintaining uniform pressure after removal of any excess blood that might be present at the investigated site. Background measurements were recorded with the laser turned off prior to each tissue measurement. Six spectra were acquired at each site with an integration time of 300 ms and averaged. In each case, visual inspection by the attending surgeon determined the tissue type corresponding to the acquired spectrum; the level of confidence in the surgeon's identification of each tissue was noted as high, medium, or low. All sites rated as low confidence by the surgeon were excluded from analysis. Visual inspection therefore served as the gold standard of detection unless the investigated site was excised, in which case spectra were correlated with histology. In each of the 21 patients studied, histology was obtained from either the parathyroid or the thyroid, or both, depending on patient diagnosis and related surgical resection. In total, histology was obtained from 16 excised thyroid samples and 10 excised parathyroid samples and found to validate the anatomical identity of the measured gland.

Near-infrared fluorescence spectra were processed using MATLAB (Mathworks Inc., Natick, Massachusetts). First, background was subtracted and the data was corrected for the wavelength dependent response of the system with a National Institute of Standards and Technology calibrated light source. Calibrated spectra were smoothed with a moving average filter of size 10 and then normalized to the maximum intensity of the mean thyroid spectrum from that patient.

In each patient, fluorescence from the parathyroid was compared to the fluorescence from the thyroid and other tissues in the neck. Figure 1(a) shows the NIR fluorescence spectra from a typical patient. The fluorescence intensity of the parathyroid was found to be the strongest among measured tissues. Further, thyroid fluorescence is observed to be stronger than the surrounding muscle and fat but weaker than the parathyroid. Figure 1(b) presents the fluorescence spectra from parathyroid and thyroid tissues, normalized to their respective peak intensities demonstrating the similarity in their spectral line shape. Since no fluorescence (intensity and line shape above the level of noise) was measured from the muscle, fat, and trachea, these signals were excluded from further analysis.

Average peak intensity for parathyroid fluorescence was consistently greater than that of the thyroid and other tissues in all

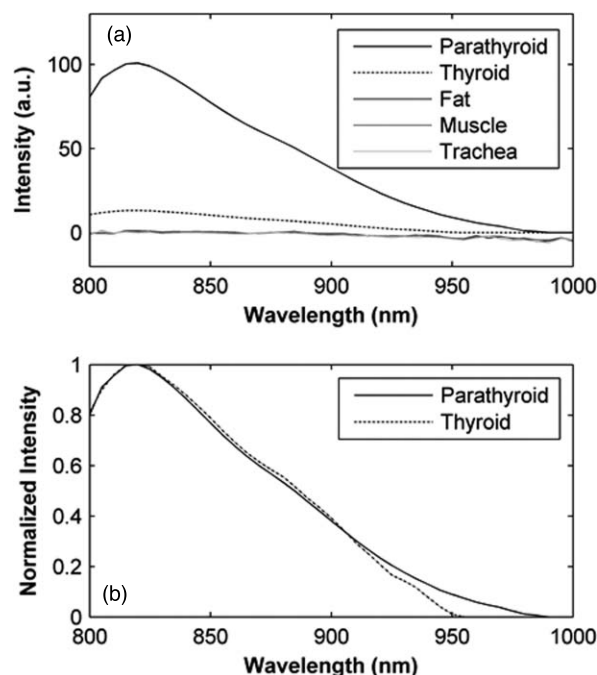


Fig. 1 (a) Fluorescence spectra measured from parathyroid, thyroid fat, muscle and trachea of a typical patient. (b) Fluorescence spectra from parathyroid and thyroid tissue normalized to their respective peaks.

21 patients [Fig. 2(a)]. The *in vivo* parathyroid fluorescence was observed to be two to eleven times more intense than the thyroid fluorescence [Fig. 2(b)]. Analysis by student's t-test shows that the parathyroid exhibits more intense fluorescence than thyroid tissue with a p-value of 0.0000235 at a 99.9% level of significance indicating that the difference in intensity is statistically significant.

Both parathyroid and thyroid histology was available in the same patient in four of the 21 cases studied due to concurrent disease. In these four patients, the fluorescence intensity of the parathyroid was found to be two to ten times greater than that of the thyroid, which is consistent with the observed results in the rest of the patients. Thus, these four patients provide histological validation to the increased intensity of the parathyroid as compared to the thyroid. It should be noted that either parathyroid or thyroid histology was available in all other patients, confirming the anatomical identity of that gland. Spectra from glands that were not excised (where identification relied on visual inspection) were found to match the spectral characteristics of the histologically correlated parathyroid and thyroid autofluorescence signals.

The 21 patients enrolled in this study represent a variety of disease states. The first seven patients in Fig. 2 were diagnosed with primary hyperparathyroidism, having either parathyroid disease (patients 1 to 3) or parathyroid disease with concomitant thyroid nodules or goiter (patients 4 to 7). The remaining 14 patients presented with thyroid disease and apparently normal parathyroids. In all 21 patients, the parathyroid fluorescence intensity was found to be consistently greater than that of the thyroid indicating that both normal and hyperfunctioning parathyroid tissue produce a much stronger fluorescence.

Results presented here show that NIR fluorescence can successfully detect parathyroid tissue *in vivo*, in real-time and

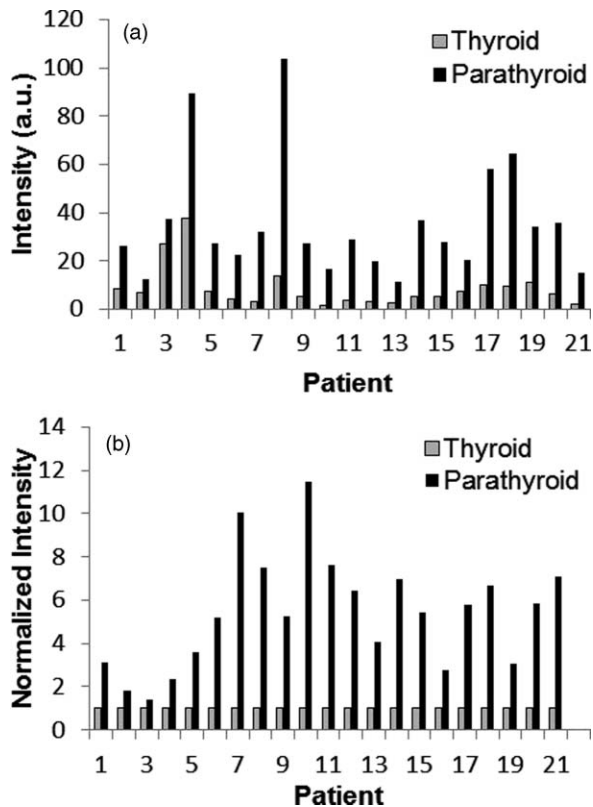


Fig. 2 (a) Average fluorescence peak intensity from parathyroid and thyroid tissues in each patient. (b) Normalized peak intensity from parathyroid and thyroid measurements from each patient. Each measurement is normalized to the average peak of all thyroid measurements in that patient. Patients 1 to 7 represent patients diagnosed with parathyroid disease; patients 8 to 21 have normal parathyroid glands.

noninvasively during endocrine surgery with near-perfect accuracy. This method improves on the sensitivity and specificity of visual recognition, a highly subjective measure that is dependent on the experience of the surgeon. More importantly, NIR autofluorescence can be used to identify parathyroid glands, regardless of thyroid or parathyroid disease. This is a major advantage over current intra-operative localization methods such as radio-nucleotide uptake, ultrasound, and iPTH assay, which are only effective when the parathyroid is hyper-active.⁹

Tissue autofluorescence is typically observed in the ultraviolet-visible (UV/VIS) wavelength range.¹² However, studies performed across multiple tissues *in vitro* (unpublished data) as well as *in vivo* (presented here) indicate that the near-infrared spectra measured from thyroid and parathyroid tissues are repeatable (within the same tissue) and highly reproducible (across all patients) reinforcing the validity of the detected signal. The observed signal exhibits the typical Stokes' shift associated with fluorescence and the peak emission wavelength does not vary, indicating that this is a form of luminescence and is most likely due to tissue autofluorescence. Studies also show that the observed signal is not an effect of the system or its various components.¹³ In particular, changing the long-pass filter does not affect the signal intensity or shape ruling out artifacts arising from the filters' transmission characteristics. Additionally, optical properties in this region of the spectrum were found to be fairly uniform between parathyroid and

thyroid tissues indicating that the effect is not explained by differences in scattering or absorption. These observations lead to the conclusion that the signal is indeed due to tissue autofluorescence; the basis for this fluorescence is, however, presently unknown.

Near-infrared wavelengths are considered the optical window and are attractive in biomedical applications due to their increased penetration depth and decreased scattering and absorption in tissues relative to UV/VIS wavelengths. This makes the NIR region optimal for biological studies spurring research efforts to use NIR wavelengths in the diagnosis and detection of disease.¹⁴ Research in NIR fluorescence has mostly involved exogenous contrast agents, most commonly polymethines.¹⁵ Notably, indocyanines, such as indocyanine green (cardio-green) have been extensively used.¹⁵ However, contrast agents are difficult to translate to the clinic due to potential problems such as toxicity, photobleaching, and localization.

Autofluorescence uses biological fluorophores that naturally occur in tissues and thus negates the need for the introduction of exogenous agents, however, tissue typically exhibits peak autofluorescence in the UV/VIS wavelengths (400 to 700 nm).¹² Except, it is well documented in the Raman spectroscopy literature that tissue spectra measured at 785 or 830 nm excitation display residual broadband signal that is believed to be fluorescence background. Several groups have exploited this broadband signal to assist in the detection of a variety of pathologies ranging from cutaneous melanin in pigmented skin disorders to neoplastic breast tissue.^{16–19} The tissue autofluorescence in these studies was attributed to fluorophores such as melanin and porphyrins but none of these studies documented peak fluorescence in the NIR region.^{16–19}

Intrinsic biological fluorophores are typically reported to exhibit peak fluorescence below 800 nm of the NIR region.¹² However, this paper clearly demonstrates the consistent presence of autofluorescence with peak emission at 820 nm in parathyroid and thyroid tissues. Das et al. used Raman spectroscopy to examine parathyroid pathology but used 830 nm excitation, thus missing the fluorescence peak.²⁰ Since the peak fluorescence emission from the parathyroid and thyroid occurs at the same wavelength, it is hypothesized that the same fluorophore is responsible. Potential candidates are likely to be present in both the thyroid and parathyroid glands. The increased fluorescence in the parathyroid implies that they would occur in greater amounts or concentrations in the parathyroid or that the fluorescence is somehow quenched in the thyroid. Porphyrins are known to be the longest emitting fluorophores in biological tissues with peak emission in the 600 to 700 nm range, however, the fluorescence shown here has peak emission above 800 nm.¹² Melanin was hypothesized to be the primary contributor to the observed NIR autofluorescence in the eye and skin, but melanin is not known to be present in parathyroid and thyroid tissues.^{17,18} A potential candidate based upon physiological examination is the calcium-sensing receptor, which is present in both parathyroid and thyroid tissues but nowhere else in the neck. Ultimately, detailed analysis beyond the scope of this initial feasibility study will need to be performed to determine the responsible fluorophore(s).

In conclusion, this paper presents the potential of using NIR autofluorescence for the real-time anatomic guidance of endocrine surgery. Even though the basis for this

fluorescence is not understood, the intensity of the measured signal allows for the feasibility of an imaging approach increasing the likelihood of its successful implementation in the operating room. Translation of this technology would reduce the rate of complications from accidental or incomplete removal of parathyroid tissue; anatomical guidance would also decrease operative time especially during lengthy parathyroidectomies where the surgeon must search for parathyroid glands.

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