Abstract

The variable location and indistinct features of parathyroid glands can make their intraoperative identification challenging. Currently, there exists no routine use of noninvasive imaging methods to enhance localization during surgery. This study demonstrates a novel realization of temporally dependent measurements of tissue autofluorescence that allows the acquisition and differentiation of specific tissue properties. In this study, parathyroid glands and surrounding tissues were collected intraoperatively, and their fluorescence decay images were acquired using FIFDRT. Analysis of FIFDRT images revealed microspectroncharacterization sufficient for tissue type identification consistent with histology \( p < 0.05 \). FIFDRT is capable of efficiently distinguishing parathyroid tissue from adjacent tissues. Such an intraoperative tool could be powerful, enabling surgeons to more efficiently identify lesions and preserve healthy tissue, subsequently improving patient outcomes.

Introduction

Primary hyperparathyroidism leads to elevated parathyroid hormone (PTH) levels and resultant hypercalcemia. \(^1,^2\) The variable location and indistinct external features of parathyroid glands can make their intraoperative identification challenging, especially when distinguishing them from adjacent fat or lymphatic tissue. \(^3,^4\) Complications like hypocalcemia and recurrent laryngeal nerve injury are generally limited, but revision surgery and comprehensive explorations can increase their risk. \(^1,^5,^6\)

Several pre-operative imaging studies are available. \(^7\) However, real-time imaging methods that can localize parathyroid gland tissue in vivo would be invaluable. Tissue autofluorescence (AF), taking advantage of differences in endogenous fluorophores, has shown promise in providing contrast that can be used to collect spatially resolved information in large field-of-views. \(^8,^9,^10\) FIFDRT extracts relative fluorescence decay information by utilizing illumination pulse shaping to produce contrast between fluorophores of different decay rates. FIFDRT utilizes safe light-emitting diodes (LEDs) to acquire two images; the first image is taken during the excitation of the tissue with light (calibration image). The second image is taken during fluorescence (decay image). A relative fluorescence decay map is created by dividing the decay image by the calibration image, and the resulting pixel values are proportional to the aggregate fluorescent decay time. A color diagram that provides contrast between parathyroid glands and adjacent tissues is generated, potentially serving as surgical map.

Methods and Materials

Patient Selection: Following approval by the UCLA institutional review board, patients were identified on a prospective basis. 81 patients and 127 individual samples were included in this study. (Table 1)

Procedure: Upon excision, specimens were sectioned and imaged using FIFDRT. Blocks were oriented with reference to the macroscopic images and FIFDRT maps to correlate histopathology and imaging.

FIFDRT Instrumentation, Protocol, and Statistical Analysis: The system, consisting of UV-LEDs and an intensified CCD camera, captured images of the fluorescence decay signatures. \(^11\) The obtained fluorescence images were normalized with each other by dividing the decay image with the calibration image. FIFDRT and visible images were automatically co-registered, as they were acquired from the same aperture. Using Matlab, ROIs were drawn on the visible images. The pixels contained in the ROIs were then grouped by tissue type and evaluated for statistical significance (Figure 1).

Table 1. Tissue Types. This table shows the categorization of various tissue types collected and imaged by the FIFDRT system. A total of 127 specimens were collected.

<table>
<thead>
<tr>
<th>Tissue Type</th>
<th>Samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>Parathyroid</td>
<td>66</td>
</tr>
<tr>
<td>Fat</td>
<td>43</td>
</tr>
<tr>
<td>Thymus</td>
<td>9</td>
</tr>
<tr>
<td>Thyroid</td>
<td>9</td>
</tr>
</tbody>
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Figure 2. (a) Computation of relative lifetime for each tissue type as a function of wavelength. (b) Computation of p-values displayed as a Manhattan plot.

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References

- The obtained fluorescence images were acquired using a method that characterizes tissue properties. FIFDRT is capable of efficiently distinguishing parathyroid tissue from adjacent tissues. Such an intraoperative tool could be powerful, enabling surgeons to more efficiently identify lesions and preserve healthy tissue, subsequently improving patient outcomes.

Results

The average relative lifetimes of different tissue types as a function of wavelength are shown in Fig2(a). Linear mixed effects models were fitted to the mean values from each tissue type (fat, parathyroid, thyroid) for a given wavelength, and a tissue type by wavelength interaction effect is shown in Fig2(b). A random effect was included to account for repeated measurements. Models allowed for heterogeneous residual variances across wavelengths. Differences in mean values between tissue types for given wavelengths were estimated using model contrasts. The analysis was adjusted for multiple comparisons to evaluate significance at a 5% false discovery rate. All statistical analyses were performed using SAS version 9.4 (SAS Institute, Cary, NC).

The computed p-values are plotted in Fig2(b) in negative log10 scale where a p<0.05 was labeled as significant. Fig2(c) displays the style of a Manhattan plot where smaller p-values (increased significance) are mapped to larger bars in the bar plot. Further, the 0.05 threshold is denoted with the gray shaded area in Fig2(b). All bars above the threshold are labeled significant.

The p-values for the parathyroid-fat, parathyroid-thyroid, and parathyroid-thyroid pairs are denoted with the red, green, and blue bar respectively. Significance was observed between parathyroid and non-parathyroid for all ten band-pass filters. In general, the middle wavelength filters (465nm-594nm) provided better quantitative contrast between thyroids and parathyroid, while all wavelengths provided statistically significant contrast between parathyroid vs fat and parathyroid vs thyroid.

Discussion

This study provides ex vivo data that has implications for making FIFDRT a reliable in vivo technique to produce a "relative decay map" of tissues, depicting analyzable color contrasts that correspond to parathyroid gland location. FIFDRT utilizes safe light and precludes the use of invasive dye\(^\text{11,16}\) or radioactive material injection, avoiding the concerns for allergic reactions or radiation exposure. \(^17\) The process of imaging and map formulation takes less than two minutes and is significantly faster than intraoperative frozen section analysis, a process that not only adds 20-30 minutes to anesthetic time but can cost $500 per sample. \(^11,18\) Furthermore, FIFDRT has the potential to help identify and therefore perhaps avoid the inadvertent removal of normal parathyroid gland during challenging thyroidectomies. Accidental removal of healthy parathyroid glands during neck dissections can cause transient hypocalcemia in 20-30% of cases, while permanent hypoparathyroidism may lead to lifelong calcium supplementation. \(^19,20\)

The basis of tissue contrast in the FIFDRT parathyroid images is likely correlated to the increased calcium content of the parathyroid hormone\(^21\) and the presence of hormone specific proteins and amino acids. The autofluorescence and lifetime fluorescence of these substances have not yet been investigated in the context of parathyroid tissues, but they have been studied extensively as isolated samples and support the utilization of calcium and parathyroid hormone specific proteins as the basis for imaging contrast. \(^22\)

Conclusions

This study provides the foundation for in vivo studies that institute FIFDRT as an intraoperative instrument that can guide parathyroid gland detection. This technology extracts relative fluorescence decay information using simple mathematical operations that allow the processing of an entire image with good contrast at video rate. This study provides preliminary data that has implications for making FIFDRT a reliable technique to produce a "relative decay map" of the neck tissues, depicting analyzable color contrasts between parathyroid glands and other tissues.