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Discriminating benign from malignant thyroid nodules in real-time using a novel probe with an integrated elastic scattering spectroscopy biopsy syringe

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DISCRIMINATING BENIGN FROM MALIGNANT THYROID NODULES IN REAL-TIME USING A NOVEL PROBE WITH AN INTEGRATED ELASTIC SCATTERING SPECTROSCOPY BIOPSY SYRINGE

by

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DEDICATIONS

This thesis is dedicated to my family, who has supported me through all my endeavors.

Mom, Dad, Christina, Lena, and Anthony – you have been a constant source of inspiration to me, and have equipped me with an insatiable drive to tackle life’s challenges with dedication and enthusiasm. Without your persistent love and support, this thesis would not have been possible.
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NICHOLAS JOSEPH GIORDANO

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ABSTRACT

Background. Thyroid cancer is the most common endocrine malignancy. The current standard of diagnosis, fine needle aspiration biopsy (FNAB) yields indeterminate results for approximately 10–25% of biopsies, necessitating thyroidectomy for diagnosis. Elastic scattering spectroscopy (ESS) is a minimally invasive optical biopsy technique that is sensitive to cellular and subcellular morphological features. We hypothesized that it was feasible to use ESS in vivo to improve our ability to preoperatively differentiate benign from malignant thyroid nodules.
Methods. Under an IRB approved protocol, we collected ESS data from patients undergoing thyroid FNAB using our miniaturized integrated ESS/biopsy probe. Spectral findings were compared to cytology.

Results. 108 patients enrolled, 5 patients were excluded due to procedural and mechanical issues. Data from 103 patients was submitted for analysis. Initial evaluation of spectra data demonstrates adequacy and comparability of the miniaturized probe to the prior full-size ESS fiber construct.

Conclusion. Performing a clinical trial using a miniaturized integrated ESS/biopsy probe in vivo is feasible and acceptable to patients. Both spectral data and cytologic material are adequate in the majority of patients. With further accrual and analysis, this ESS device may provide cost-effective, real-time, and operator independent assessment of thyroid nodules.
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ABBREVIATIONS

µm         micrometers
ATC        anaplastic thyroid cancer
ATC        Anaplastic thyroid cancer
BMC        Boston Medical Center
BUSM       Boston University School of Medicine
ESS        elastic light-scattering spectroscopy
FNA        fine needle aspiration
FNAB       fine needle aspiration biopsy
FTC        Follicular thyroid cancer
FTC        follicular thyroid cancer
IRB        Institutional Review Board
MTC        Medullary thyroid cancer
MTC        medullary thyroid cancer
NIH        Nation Institute of Health
nm         nanometers
NPV        negative predictive value
PCA        principal component analysis
PPV        positive predictive value
PTC        Papillary thyroid cancer
PTC        Papillary thyroid cancer
<table>
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<tr>
<th>Acronym</th>
<th>Description</th>
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<tr>
<td>SVM</td>
<td>support vector mechanisms</td>
</tr>
<tr>
<td>T₃</td>
<td>triiodothyronine</td>
</tr>
<tr>
<td>T₄</td>
<td>thyroxine</td>
</tr>
<tr>
<td>TSH</td>
<td>thyroid stimulating hormone</td>
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INTRODUCTION

The Normal Function of the Thyroid Gland

The thyroid gland, also referred to as the thyroid, is anatomically located in the neck, just below the laryngeal prominence, or what’s more commonly referred to as the “Adam’s apple.” The thyroid is a bi-lobed, butterfly-shaped organ that is connected by an isthmus that wraps around the larynx.

The thyroid has a wide range of functions in the human body, and is one of the largest endocrine glands. Thyroid functions are critical in controlling the body because the thyroid regulates metabolic rates, proteins, and other hormones in the body. The main products of the thyroid gland are two hormones: triiodothyronine (T\textsubscript{3}) and thyroxine (T\textsubscript{4}). These hormones principally affect the regulation of metabolism within the body.

The production of T\textsubscript{3} and T\textsubscript{4} is regulated by another hormone in the body, thyroid stimulating hormone (TSH). There is a negative-feedback loop between the amounts of TSH and T\textsubscript{4} production to regulate normal body functions.

Thyroid Disorders

There are three main thyroid disorders:

1) Hyperthyroidism – an overactive thyroid
2) Hypothyroidism – an underactive thyroid
3) Thyroid Nodule – a thyroid neoplasm

Hyperthyroidism is caused from an overproduction of the main thyroid hormones, T\textsubscript{3} and T\textsubscript{4}. A lack of the negative feedback mechanism which would normally inhibit the
production of thyroid hormones is typically present. Hypothyroidism occurs when not enough T3 and T4 is naturally produced by the body. A thyroid nodule indicates a thyroid neoplasm, but is usually found to be benign. All three of these thyroid disorders may present with an enlarged thyroid, otherwise referred to as a goiter.

**Thyroid Nodules**

At 60 years of age, approximately 50% of the population have at least one thyroid nodule. The clinical spectrum of a presenting thyroid nodule can range from a small, singular incidental nodule, or a large, symptomatic, pressure-inducing mass that can take up the near entirety of a thyroid lobe.

**Thyroid Cancer**

Approximately 5% of thyroid nodules are found to be malignant. There are four types of thyroid cancer:

1) Papillary Thyroid Cancer
2) Follicular Thyroid Cancer
3) Medullary Thyroid Cancer
4) Anaplastic Thyroid Cancer

Papillary thyroid cancer (PTC) is by far the most common of all thyroid cancers, occurring in approximately 80% of the cases. Females are three times as likely to be diagnosed with PTC and it is usually not an aggressive cancer. Diagnosis of this particular form of cancer typically occurs between the ages of 30 and 50 years of age.
Follicular thyroid cancer (FTC) makes up approximately 15% of all thyroid cancers. Just like PTC, females are three times more likely to be diagnosed with this particular cancer. This particular type of cancer can be more aggressive, especially in older patients. FTC is typically diagnosed in individuals from 40 to 60 years of age.

Medullary thyroid cancer (MTC) occurs in approximately 10% of all thyroid cancers. This form of thyroid cancer is found to have an incidence equal in the female and male population, and is found to have a 50% five-year survival rate for stage IV cancer. MTC is often diagnosed in patients between 40 and 50 years of age.

Anaplastic thyroid cancer (ATC) is rare, occurring in less than 5% of thyroid nodules. It is found to have a higher occurrence in females and is the most invasive and aggressive form of thyroid cancer. The five-year survival rate with ATC is approximately 7%, and is typically diagnosed in patients of the age of 65.

Thyroid cancer is the most common form of endocrine malignancy in the United States. An estimated 60,000 new cases of thyroid cancer will be diagnosed in 2013. Incidence rates of thyroid cancer have been growing by 7% each year. These growing rates of thyroid cancer may be due to advancements in imaging techniques such as neck ultrasonography. With an increase in incidence, a stable mortality rate has been seen in thyroid cancer.
Diagnosing Thyroid Cancer

Current state-of-the-art technology in endocrinology utilizes a diagnostic test called a fine needle aspiration (FNA) biopsy in order to diagnose a thyroid nodule to determine whether the tissue is malignant or benign. A FNA is comprised of a non-sterile procedure in which a patient has a series of biopsy needles inserted into their thyroid in accordance with the Bethesda standard. Each needle is used to collect cells from the thyroid, and those cells are then sent to a local pathology process where a pathologist will analyze the cells in a cytology report. After three to five days, the cells will be placed in one of four categories: benign, indeterminate, insufficient or malignant. The following criteria were depicted for categorizing which cytology results were placed in each of the four categories of a fine needle aspiration biopsy (FNAB). The table is reprinted below for convenience.
**Table 1:** A table from the Bethesda System for Reporting Thyroid Cytopathology. The table describes the recommended diagnostic criteria for each category of FNA cytology. This figure is taken from Ali et al., 2009.

<table>
<thead>
<tr>
<th>Category</th>
<th>Diagnostic Criteria</th>
</tr>
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<tbody>
<tr>
<td>I. Nondiagnostic or Unsatisfactory</td>
<td>Cyst fluid only&lt;br&gt;Virtually acellular specimen&lt;br&gt;Other (obscuring blood, clotting artifact, etc)</td>
</tr>
<tr>
<td>II. Benign</td>
<td>Consistent with a benign follicular nodule (includes adenomatoid nodule, colloid nodule, etc)&lt;br&gt;Consistent with lymphocytic (Hashimoto) thyroiditis in the proper clinical context&lt;br&gt;Consistent with granulomatous (subacute) thyroiditis&lt;br&gt;Other</td>
</tr>
<tr>
<td>III. Atpyia of Undetermined Significance or Follicular Lesion of Undetermined Significance</td>
<td>Specify if Hurthle cell (oncocytic) type</td>
</tr>
<tr>
<td>IV. Follicular Neoplasm or Suspicious for a Follicular Neoplasm</td>
<td>Suspicious for papillary carcinoma&lt;br&gt;Suspicious for medullary carcinoma&lt;br&gt;Suspicious for metastatic carcinoma&lt;br&gt;Suspicious for lymphoma&lt;br&gt;Other</td>
</tr>
<tr>
<td>V. Suspicious for Malignancy</td>
<td>Papillary thyroid carcinoma&lt;br&gt;Poorly differentiated carcinoma&lt;br&gt;Medullary thyroid carcinoma&lt;br&gt;Undifferentiated (anaplastic) carcinoma&lt;br&gt;Squamous cell carcinoma&lt;br&gt;Carcinoma with mixed features (specify)&lt;br&gt;Metastatic carcinoma&lt;br&gt;Non-Hodgkin lymphoma&lt;br&gt;Other</td>
</tr>
</tbody>
</table>

The benign category of cytology is by far the most common. A graph below demonstrates the average findings of the FNA biopsy.
Figure 1: A graph demonstrating the typical occurrence of each of the four main categories of a thyroid FNA biopsy.

The diagnostic accuracy of a FNA not only depends on the clinical sensitivity of the test, but also on the administrator. The insufficient category of FNA biopsy is a disputed area in cytological diagnosis of patients with thyroid lesions, because there are inherent similarities in cells under a light microscope. It has been found that by skillfully applying the techniques of an FNA biopsy, in conjunction with the recovery of an adequate sample, and a decreased level of interpretive errors, the number of patients diagnosed with an indeterminate thyroid nodule can be decreased.

Risk management of a thyroid nodule is guided by the results of a FNA biopsy. Below is the standard of care associated with the results of a thyroid biopsy.
Table 2: A table from the Bethesda System for Reporting Thyroid Cytopathology. The table is shown here for reference in determining the typical classification of a FNA biopsy, its risk of malignancy, and the typical management used with each classification. This figure is taken from Ali et al., 2009.

<table>
<thead>
<tr>
<th>Diagnostic Category</th>
<th>Risk of Malignancy (%)</th>
<th>Usual Management</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nondiagnostic or Unsatisfactory</td>
<td>1-4</td>
<td>Repeat FNA with ultrasound guidance</td>
</tr>
<tr>
<td>Benign</td>
<td>0-3</td>
<td>Clinical follow-up</td>
</tr>
<tr>
<td>Atypia of Undetermined Significance or Follicular Lesion of Undetermined Significance</td>
<td>~5-15</td>
<td>Repeat FNA</td>
</tr>
<tr>
<td>Follicular Neoplasm or Suspicious for a Follicular Neoplasm</td>
<td>15-30</td>
<td>Surgical lobectomy</td>
</tr>
<tr>
<td>Suspicious for Malignancy</td>
<td>60-75</td>
<td>Near-total thyroidectomy or surgical lobectomy</td>
</tr>
<tr>
<td>Malignant</td>
<td>97-99</td>
<td>Near-total thyroidectomy</td>
</tr>
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For an indeterminate biopsy, the patient undergoes surgery for therapeutic reasons. Over 66% of thyroid surgeries are done simply for diagnostic purposes.\(^9\) FNA biopsy is a great screening test for cancer, but has a rather high rate of false positives (indeterminates) which leads to a high number of diagnostic surgeries.\(^9\) If there was a way to take out the margin of error in a FNA biopsy, and provide a more accurate diagnosis of thyroid cancer, thousands of dollars in surgical costs could be saved, and an increased quality of life could be achieved for the patient.

**Elastic Scattering Spectroscopy**

Elastic Scattering Spectroscopy (ESS) is an optical imaging technique which uses broadband light to take measurements. The light is in the visible spectrum from 300 – 800 nanometers (nm) and is non-ionizing with no known negative side effects.\(^12\) ESS has been used in other organs and tissues in the body including, but not limited to the esophagus, oral cavity lesions, breast tissue and colonic polyps.\(^13\)–\(^23\) In all of these tissues,
ESS has found to accurately distinguish benign from malignant spectra. ESS is capable of providing an objective assessment of tissue that is user independent, and does not require the insight of a pathologist. ESS also has the additional benefits of being portable, and is capable of taking measurements in real-time.

ESS works by emitting a point measurement. A graphic below depicts the basic functions of ESS.

**Figure 2:** A graphic demonstrating the scattering of light upon interaction with a tissue of interest. In this case, the tissue is the thyroid.

A pair of fiber optics are placed in contact with a tissue of interest, in this case, the thyroid. A light source then emits a photon which travels through a fiber optic and interacts with the thyroid. In the tissue the photon does one of two things: it is either absorbed into the tissue in which case we never see it again; or the photon is scattered
about in the tissue. In the case in which the photon is scattered, eventually a photon will reach a second fiber optic, deemed the detector fiber, which carries the photon back to a spectrometer where the photon is detected. The photon carries with it information about its traveled path, and when compiled and displayed on a computer, this displays a spectrum. The spectrum is a visual representation of the matter that the photon traveled through. This spectrum represents that point measurements that ESS took, and is directly affected by the size and density of the organelle with which the photon interacted.

ESS is capable of performing an “optical biopsy” because this optical method is sensitive to most of the features that pathologists look for under the microscope. Cellular and sub-cellular components like the nucleus, nucleolus, and mitochondria are represented and accounted for in the ESS optical spectrum. Through studying ESS in other organs and tissues it has been found that ESS is sensitive to both cellular and subcellular morphological features and therefore features associated with malignant transformation are detected by ESS.13

FNA is a great screening tool for malignant nodules in the thyroid and has a very high sensitivity for malignant thyroid tissue.24 However, as seen in figure 1, 35% of thyroid nodules will undergo thyroid surgery, even though only 5% of those nodules harbor a malignant tissue. The majority of thyroidectomies and lobectomies are done purely for diagnostic reasons, because of low specificity and positive predictive values of the current state-of-the-art diagnostic tool in endocrinology, FNA biopsy.25

In other human organs and tissues, it has been found that ESS has a high specificity when detecting benign from malignant tissue. It is therefore proposed in this
research study that we integrate FNA biopsy with an ESS probe in order to create the ideal diagnostic tool for detecting thyroid cancer. We hypothesized that an integrated ESS biopsy syringe will minimize the amount of diagnostic surgeries done on the thyroid, especially for patients who have an indeterminate result from an ordinary FNA.

In order to test this hypothesis that ESS would have a high specificity in detecting benign from malignant thyroid tissue, a small *ex vivo* clinical trial was conducted at Boston Medical Center (BMC) in Boston, MA. The clinical trial consisted of 36 thyroid samples which were measured with ESS within ten minutes of surgical removal. The study revealed that ESS had a 95% specificity with this small sample size.\(^{24}\)

Using this *ex vivo* study as motivation, a larger *in vivo* validation study was organized to confirm the assumption set forth in the *ex vivo* trial and see if the findings would be consistent if studied in perfused tissue.

The study prompted some major design changes from the probe used in the *ex vivo* clinical trial. The optical probe had to be engineered around the concentric constrains of a 23-gauge needle typically used during a FNA biopsy. This meant the optical geometry of the probe had to be changed, and the diameter of the fiber optics used for the emission and detection of a photon had to be reduced. With the reduction in size came a quadratic reduction in cross-sectional area. This meant that in order to emit the same intensity light used in the *ex vivo* study, a longer acquisition time had to be used to acquire the measurements. Although longer, both the larger ESS probe used in the *ex vivo* study and the smaller, *in vivo* optical probe took measurements in under one second.
SPECIFIC AIMS

The specific aims of this project can be broken up into four main objectives:

1) Conduct an in vivo clinical trial
2) Miniaturize the ESS probe for a 23 gauge needle
3) Co-register ESS readings with cytology and histology
4) Use data to discriminate benign & malignant nodules through automated feature analysis

This project is an expansion of the work done by Suh et al who has established that an ex vivo model of this probe can accurately distinguish between benign and malignant thyroid tissue. This was done with a large, 200 µm source and detector. Our first objective with this research is to conduct an in vivo clinical trial with an ESS probe, and test if similar findings of ex vivo thyroid tissue can be seen with perfused tissue.

In order to conduct an in vivo study, the ESS optical probe must be made to fit through the concentric constraint of a standard 23 gauge biopsy needle. The fiber optics used in the ex vivo study were too large to meet the constraint of a biopsy needles, so the ESS probe needed to be miniaturized.

When we have obtained data from the miniaturized in vivo ESS probe, we will have to co-register ESS readings with cytology and histology because we want to correlate malignant, perfused thyroid tissue with its respective and unique ESS optical signature. That way, we can establish and eventually recognize malignant thyroid tissue based off of its optical signature.
Lastly, we would like to use the ESS data to discriminate benign from malignant nodules through an automated feature analysis. In order to automate this feature analysis we need to firmly establish what a malignant spectra looks like by entering and correlating approximately 20 malignant thyroid tissues into the study.
MATERIALS AND METHODS

Elastic Scattering Spectroscopy System

The ESS instrumentation consists of a light source, in this case, a pulsed xenon arc lamp, an optical fiber probe, a spectrometer, and a computer to control the various components and record the spectra. These pieces of equipment are housed in a small and portable unit, seen below in figure 3.

Figure 3: An image of the portable ESS console.

When a technician presses a button on the ESS console, a short pulse (~7 ms) of white light is emitted from the xenon arc lamp (Perkin Elmer, Inc., Fremont, CA) and travels through a pair of flexible fiber optic probes. The optical fiber which carries the light from the source to the tissue is deemed the source fiber. The optical fiber which
carries the light scattered from the tissue back to the spectrometer is deemed the detector fiber. A graphic is shown below for clarification.

**Figure 4:** A graphic of the basic setup of our ESS optical probe. The essential components, a computer, xenon lamp, and spectrometer are housed within one portable unit. A pair of fiber optics connects the ESS equipment to the biopsy syringe.

![Diagram of ESS optical probe setup](image)

The photons emitted from the source fiber hold one of two fates when they reach thyroid tissue. The photons are either absorbed in the thyroid tissue, or scattered about the tissue. In the instance where the photon is scattered about, eventually some of the photons will reach the detector fiber, travel through it, and be sensed by the spectrometer. The photon carries information with it about the size and density of the tissue it just interacted with, and will display a spectrum based on the cellular and sub-cellular components of the tissue.²⁶
It has been demonstrated in other organs and tissues that ESS is sensitive to cellular and subcellular changes in the cell, including most of the features that pathologists look for under a microscope when diagnosing a malignant tissue, as well as the features associated with malignant transformation. In this sense, ESS is capable of providing an “optical biopsy” which is capable of discovering these features in a cell, without extracting any cells or cellular matter of the tissue. For this reason, ESS does not require multiple passes with a biopsy syringe to provide an accurate diagnosis, unlike the current state-of-the-art care with the Bethesda system established in 2007.

*Designing the Miniaturized In Vivo ESS Probe*

Before the probe was miniaturized, theoretical and physical measurements were done to study the effect that the change in fiber optic probes would have on the scattered light in tissue. Design priority was given to maintaining the intensity of light transmitted through the fiber optics; thus, the source fiber optic was designed to be larger than the detector fiber. Through a Monte Carlo simulation of the Mie theory, analysis showed that with the concentric constraint of a 23 gauge biopsy syringe, which is found to have an approximate diameter of 0.34 mm, it is best to use a 150 µm source fiber, and a 100 µm detector fiber. With the smaller fibers used, the cladding, buffer and jacket of the fiber were found to be thinner, decreasing the center-to-center distance between the fiber optic probes, and making the ESS probe more sensitive to scattering events in the tissue. This was an added benefit found during simulation, which enables the *in vivo* optical probe to have an increased sensitivity to scattering events.
A smaller optical geometry was designed for the concentric constraint of a 23 gauge biopsy needle. A 150 micron source and a 100 micron detector were selected for the optical fibers of the \textit{in vivo} probe. Together, the fibers had a 163 micron center-to-center distance, and were housed in a polyimide casing (Kapton™) that is approximately 360 microns in diameter.

A lightweight biopsy syringe was then designed and engineered to house the fiber optics of the ESS probe while still maintaining the functionality of a biopsy syringe. The unit was engineered to be used with a single hand, just like a typical biopsy syringe. The source and detector fibers were threaded through a 23-gauge needle. The integrated ESS biopsy syringe was designed for easy handling and sterilization. The collection and recording of a single spectrum takes less than one second, with the data transfer time being the limiting factor.

\textit{System Calibration}

Before any spectra of the nodules are taken, a reference spectrum was recorded. This calibrates the overall system response by recording the diffuse reflectance from a spectrally flat diffuse reflector, Spectralon™ (Labsphere, Inc., North Sutton, NH). The reference spectrum allows spectral variations in the light source, fiber transmission, spectrometer, and fiber coupling to be accounted for. Each consequent nodule or normal tissue spectrum was divided by this reference spectrum to obtain the system-independent reflectance spectrum of the site being investigated.
Miniaturized Probe Validation Study

A small (n = 16) validation study was done with sequential patients for the newly designed \textit{in vivo} optical geometry. For this experiment, the miniaturized optics (150 micron source, and 100 micron detector) were used in an \textit{ex vivo} study under the same protocol as the previously published \textit{ex vivo} ESS optical probe experiment.\textsuperscript{1} The study was approved by the Institutional Review Board (IRB) of Boston Medical Center and informed consent was obtained prior to their participation from patients with thyroid lesions already scheduled to undergo thyroidectomy. ESS data was collected from sequential patients scheduled for surgical thyroidectomy, using immediate \textit{ex vivo} thyroidectomy specimens in the frozen section pathology room within 5–10 min of surgical removal and prior to the formalin fixation. Specimens were bisected by a pathologist at site, and ESS readings were performed on either the most prominent nodule or preoperative FNA examined nodules. All nodules were bivalved, and spectra were obtained from five sites with five repetitive readings per site per nodule on average, depending on the size.

Normal pathological processing occurred from this point forward. After pathological diagnosis has been made, the subjects are de-identified, and the optical spectra findings are correlated with the histological criteria for indeterminate thyroid, benign thyroid, and thyroid cancer.
Data Collection and Spectral Acquisition of the In Vivo Clinical Trial

For the *in vivo* experiment of the 150 micron source and 100 micron detector, under Boston Medical Center IRB approval, subjects were recruited (*n* = 103) from the Thyroid Clinic at Boston University School of Medicine (BUSM), which is located in Boston Medical Center (BMC) under the direction of Dr. Stephanie Lee. Patients undergoing a standard FNA of their thyroid gland as a part of their normal clinical care were eligible for the study. Prior to participation in the study, the endocrinologist obtained informed consent from the patient after detailing the treatment plan, risks, benefits, alternatives, cost, etc. The portable instrument was brought into the biopsy room and calibrated. The FNA biopsy syringe, with the optical probe inside of it, was placed into the neck as per standard FNA protocol, and guided into the thyroid nodule by ultrasound imaging. With the help of a technician, the researcher obtained the optical spectra by triggering the spectrometer. Five measurements were taken, over three seconds, per each unique recording site. Hemorrhage, degenerative cyst and necrotic tissues were avoided with the help of ultrasound guidance, and from real-time assessment of spectra by the technician during optical biopsy readings. After the spectral data was obtained, the optical probe was retracted while cells were aspirated into cytology tubes.

Normal pathological processing occurred from this point forward. After pathological diagnosis has been made, the subjects are de-identified, and the optical spectra findings are correlated with the histological criteria for indeterminate thyroid, benign thyroid, and thyroid cancer.
More patients were recruited in the *in vivo* study to account for the increasing amount of variables when performing *in vivo* measurements. Each patient’s specimen serves as its own control because the biopsy needle passes through normal thyroid tissue en route to the thyroid nodule, and during the *ex vivo* experiments, normal thyroid tissue was measured when available. However, spectra from normal/control tissue are not used, or needed for optical diagnosis of the suspect tissue.

*Subject Inclusion Criteria*

Subjects were of both genders, ranging from ages 23-84 years old, of all ethnic groups speaking English, or Spanish. No children were included in the study. Additionally subjects who participated in the *ex vivo* study were already undergoing a thyroidectomy for thyroid nodules, thyroid cancer and thyroid goiter with nodules. For the *in vivo* study, subjects were already undergoing a thyroid biopsy for thyroid nodules, thyroid cancer and thyroid goiter with nodules. Only patients already undergoing a biopsy for clinical purposes underwent optical biopsy at the same time as their physical biopsy. Accrual continues for the *in vivo* study at the time of this publication.

*Subject Exclusion Criteria*

Subjects with nodules less than 1 cm in size, with infectious diseases or on blood thinning medications (i.e. Coumadin) were excluded.
Spectral Processing (Co-Registration)

ESS measurements from a given patient are assigned to a malignant or benign category, based on a patient’s individual histopathology report. Each ESS optical spectrum was co-registered with the histopathology from the nodule measured. The data was then normalized to one so that a visual inspection upon ration analysis could be done on the spectra.

Just as a pathologists can detect morphological changes from visual inspection of a histopathology slide, those same morphological changes are found in ESS spectra. By training an algorithm with approximately 20 perfused malignant thyroid tissues, in vivo, we can firmly establish what a malignant spectra looks like to ESS in vivo. Once this algorithm is trained, it is expected to perform objective and automated differentiation between benign and malignant thyroid tissue in real-time.

Spectral Analysis

The raw spectrum collected from each measurement consists of 1,000 detector pixels, or data points from wavelength 187 – 873 (nm). The five measurements taken at each site were averaged to improve the signal-to-noise ratio. All spectra were then pre-processed by cropping, smoothing and normalizing before analysis. A diagnostic algorithm will be developed based on multidimensional data analysis methods to classify the measured spectra. Given the high dimensional nature of the data, a framework consisting of dimensionality reduction will be used prior to classification of benign and
malignant. Dimensionality reduction will be accomplished using principal component analysis (PCA).\textsuperscript{28} For classification, linear support vector mechanisms (SVM)\textsuperscript{29,30}, trained with the features of benign and malignant ESS spectra were extracted with PCA, and were used. Leave-one-out cross-validation was used to obtain estimates of performance in the form of sensitivities and specificities.
RESULTS

An Internal Review Board (IRB) approved clinical study was done at Boston Medical Center (BMC) in which 36 patients were enrolled. Before undergoing a thyroidectomy, patients consented to have their thyroid tissue measured with a large ex \textit{vivo} ESS probe fitted with a 200 \( \mu m \) source and detector fiber immediately after removal. The results of this trial can be seen in \textit{table 3}.

<table>
<thead>
<tr>
<th>ESS</th>
<th>Histopathology</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Malignant</td>
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<tr>
<td>Malignant</td>
<td>12</td>
</tr>
<tr>
<td>Benign</td>
<td>3</td>
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</tbody>
</table>

\textit{Table 3}: Shows the results of the \textit{ex vivo} clinical trial with the large ESS optical probe.

The \textit{ex vivo} clinical study revealed that ESS was 95\% specific in identifying benign from malignant thyroid tissue when compared against the gold standard of histopathology.\textsuperscript{24} The ESS probe was found to have a high specificity when used as a diagnostic test for thyroid cancer.

An \textit{in vivo} IRB approved clinical study was done at BMC in which 108 patients were assessed for eligibility and 103 were submitted for analysis. Three patients declined to
participate in the research and five patients were excluded from analysis. Out of the 103 participants enrolled in the study, the average enrolled patient was found to be a Caucasian female in her 50’s. 20 of the patients have undergone surgery, and 6 have had histopathology-confirmed malignant thyroid tissue.

Table 4: Demographics of the in vivo optical probe study.

<table>
<thead>
<tr>
<th>Demographics (n = 103)</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Characteristic</strong></td>
<td><strong>Result</strong></td>
</tr>
<tr>
<td>Gender</td>
<td>Female = 92, Male = 11</td>
</tr>
<tr>
<td>Age</td>
<td>Avg = 53 (Range = 23 - 84)</td>
</tr>
<tr>
<td>Race</td>
<td>White = 41, Black = 39, Asian = 6, Hispanic = 14, Other = 3</td>
</tr>
<tr>
<td>Cytology</td>
<td>Malignant = 5, Benign = 72, Indeterminate = 23, Insufficient = 3</td>
</tr>
<tr>
<td>Histopathology</td>
<td>Malignant = 6, Benign = 14, No Surgery Yet = 83, [ PTC = 3, FVPTC = 1, ATC = 2 ]</td>
</tr>
</tbody>
</table>

Of the 108 patients assessed for eligibility, three patients declined to participate in the study. After the study procedure was explained to the patients, two of the patients expressed a fear of needles, and did not want to endure the extra 10-15 seconds to take
ESS measurements. One of the patients had already participated in the study, and did not want to re-participate in the study. Five patients were excluded from the analysis of the study. On two of the patients ESS probes failed to deploy the fiber optic fiber and no spectra was attained, and on three of the patients a procedural error barred any spectra from being attained. *Figure 1* below shows a graphical representation of the *in vivo* trial enrollment.

**Figure 5:** A flowchart of the *in vivo* trial enrollment.

```
Assessed for eligibility
(n = 111)

\[\rightarrow\] Declined to participate (n = 3)

Participated in study
(n = 108)

\[\rightarrow\]

Excluded from analysis (n = 5)
- Probe failed to deploy (n = 2)
- Procedural error (n = 3)

Analysis

\[\rightarrow\]

Submitted for analysis
(n = 103)
```
**Figure 6:** Shows the plot of averages for nodules that fell into the benign, and malignant categories for the previous *ex vivo* study utilizing a 200 micrometer (µm) source and 200 µm detector.
**Figure 7**: Shows the plot of averages for nodules that fell into the benign, and malignant categories for the *ex vivo* study utilizing a miniaturized ESS probe. The probe contained a 150 micron source and 100 micron detector.
Comparison of \textit{ex vivo} 200/200 to \textit{ex vivo} 150/100

The spectra taken from these two probes were taken with different optical geometry. The \textit{ex vivo} 200/200 probe utilized a 200 \textmu m source and a 200 \textmu m detector. The \textit{ex vivo} 150/100 probe utilized a 150 \textmu m source, and a 100 \textmu m detector. There seems to be a greater differentiation between the benign and malignant spectra from the \textit{ex vivo} 150/100 probe, when compared to the \textit{ex vivo} 200/200 probe.

Comparison of \textit{ex vivo} 150/100 to \textit{in vivo} 150/100

Spectra collected from the two probes consisting of the same optical geometry have similar looking spectra. The greatest variance between benign and malignant spectra for both the \textit{ex vivo} 150/100 and the \textit{in vivo} 150/100 can be noted at the lower wavelengths from approximately 300 – 400 nm and at the higher wavelengths from approximately 600 – 800 nm.
Figure 8: Shows the plot of averages for nodules that fell into the benign and malignant categories for the in vivo study utilizing a miniaturized ESS probe. The probe contained a 150 micron source and 100 micron detector.
**Figure 9:** Demonstrates what the real-time raw data looks like when collecting measurements with the ESS *in vivo* biopsy syringe. The plot shows measurements taken in two separate regions of a nodule. Location #1 is taken in normal thyroid tissue.
Figure 10: Example data taken from a cystic thyroid nodule with the ESS in vivo biopsy syringe. The plot shows measurements are taken in three separate regions of a nodule, and show a clear differentiation between the spectra of normal and cystic tissue.
Figure 11: Example measurement of a thyroid location with hemoglobin saturation, as indicated by the large trough at approximately 420 nm.
DISCUSSION

Preliminary *in vivo* results of the ESS optical biopsy syringe has been consistent with our *ex vivo* initial trial. Upon visual inspection of the normalized spectra, one can see a differentiation between the benign and malignant spectra, especially at the lower wavelengths. Upon viewing the electromagnetic spectrum (see *figure 12* below) it is evident that the regions of greatest variability between the benign and malignant spectra occur at the lower wavelengths correlating with ultraviolet light.

*Figure 12:* A figure showing visible light in relation to the complete electromagnetic spectrum. Figure was downloaded from “what is the electromagnetic spectrum at http://scimad.com”.

The changes noted in the results to can be traced to the regions of ultraviolet light (320 to 400 nm) and are consistent with Rayleigh scattering, which assumes that photons are elastically scattered.\(^3^1\) Looking at *figures 6, 7* and *8* an increasing differentiation
between the benign and malignant spectra at the lower wavelengths is noted. The data suggests that as the optical geometry of the probe gets smaller, the probe becomes more sensitive to Rayleigh scattering.

During the miniaturization process of the probe, a smaller optical geometry was utilized. Instead of the original 200 µm source and 200 µm detector fiber used in the ex vivo probe, both a smaller source, at 150 µm and a smaller detector, at 100 µm were utilized. Not only were the inner diameters of the fibers smaller than the original probe, but the cladding, and the silicone coating of the fiber, were also thinner. This allowed the source and detector fiber to be oriented closer to one another. It has been found through both empirical and theoretical measurements that a smaller optical geometry allows for increased sensitivity to photon scattering. This could account for the greater differentiation seen with the smaller optical geometry.

Visual inspection of the averaged, normalized spectra from both the malignant and benign thyroid tissues of the in vivo ESS optical probe suggest that there is a unique optical signature associated with a specific diagnosis of thyroid cancer. Although it is too early to perform statistical analysis to increase the validity, early results of this experiment suggest that ESS is capable of accurately differentiating and diagnosing thyroid tissue in real-time.

Additional benefits of the real-time feedback that ESS provides are that it is capable of detecting the contents of a thyroid nodule, including but not limited to cystic fluid, micro and macro calcifications, and hemoglobin content. This information carries with it the potential to increase the diagnostic capabilities of an FNAB. This can ensure
that a biopsy syringe is actually inside of the nodule, which is extremely helpful when there aren’t well-defined margins on smaller, sub-centimeter nodules which require a FNAB.

While conducting the clinical trial, it was seen that the raw spectra for normal thyroid tissue (figure 9) are noticeably different than those of cystic fluid (figure 10). Cystic fluid can often be found in thyroid nodules, although in order to attain a diagnostic cytology report, a clinician must biopsy solid tissue within the nodule. These finding suggests that an ESS integrated biopsy syringe would not only be able to increase the specificity of a FNAB through analyzing the spectra for a malignant tissue, but can also increase the quality of cytology, leading to better diagnostic value for the gold standard.

Reviewing the compiled FNAB classifications of the patients entered into the study show a smaller percentage of insufficient biopsies than those reported in the literature. Looking at table 4 one can see that 3 out of 103 patients, or approximately 3% received an insufficient biopsy with the ESS integrated optical biopsy syringe, as opposed to 10%, the average number of insufficient biopsies reported in the literature.1

The ESS integrated biopsy syringe was also found to be sensitive to calcifications within nodules. Thus far in the study, only a visual inspection of the normalized data has been done. In the future, a study of the intensities of the spectra could provide more diagnostic information on the thyroid tissue biopsied with the ESS probe, and potentially differentiate between types of thyroid cancer.

ESS measures the reflection of light through absorbance units. The more light emitted from the source fiber, the more light will be reflected, regardless of the tissue
being studied. The amplitude of the spectra, in conjunction with the shape of the spectra, could tell an endocrinologist important information about the tissue of interest. It is established that calcification within or around a thyroid nodule can increase the chances of a malignant thyroid nodule, but these features aren’t always readily visible during an ultrasound. With ESS, it has been seen that calcium will reflect much more light than benign or malignant thyroid tissue. In figure 9 it was noted that location 2 was confirmed to be a site of calcification. Four times the amount of light, or pulses were emitted in the normal tissue, known to be location 1, than in location 2, the site of calcification, however both spectra utilized the full dynamic range. Therefore, by studying the amplitude of the spectra, and the amount of pulses released from the light source, ESS provides one with the ability to measure another unique and distinguishing property of tissue, absolute intensities.

Lastly, it was noted from the ESS spectra that hemoglobin saturation can be indicated by a large absorption (trough) at approximately 420 nm. For reference, figure 11 shows an example of an ESS measurement taken at a location with hemoglobin saturation. When these measurements are made, in real-time, a technician can advise an endocrinologist to move to another location within the nodule, and can increase the probability of attaining a diagnostic biopsy.

Due to the fact that there are currently six histopathology-confirmed malignancies entered into the study at the time of writing this thesis, an automated algorithm was not developed to diagnose the thyroid tissue measured with ESS. Before such an algorithm is developed, a malignant ESS spectrum must be firmly established with approximately 20
samples of perfused thyroid tissue. With such a large variation between the malignant and benign thyroid spectra seen in figure 8 the current data is encouraging, and suggests that such an automated algorithm can be easily incorporated into the data collection software. In the future, we hope that with little training, a technician can help to guide a biopsy syringe to diagnostically rich portion of the nodule, and classify a nodule from a point-measurement, filling the role of a pathologist.

Preliminary results of this study indicate that future applications of this technology would be beneficial in surgical removal of cancers of embedded, hard-to-reach organs. Surgical margins on pancreatic cancer would be one of the most logical applications for this technology to ensure in real-time that all of the malignant tissue is excised during an operation.

Manufacturing the ESS integrated optical biopsy syringe has cost approximately two hundred dollars, with one hundred and sixty dollars going towards labor in milling the parts for the probe. Upon large-scale production, labor costs would be drastically decreased. The miniaturized ESS probe was found to be easily adopted by endocrinologists in the clinic, due to the probe’s design, which is similar to that of a typical biopsy syringe. The ESS integrated biopsy syringe is also capable of being decontaminated and sterilized, and therefore can be reused.
There is an unmet clinical need for a more accurate method to diagnose thyroid cancer. Currently, there is no definitive way to determine whether a thyroid nodule is benign or malignant when cytology yields an indeterminate result. Preliminary data from this study suggests that ESS could potentially serve this unmet need in real-time.
CONCLUSION

ESS spectroscopy was found to be successfully integrated into the lumen of a 23 gauge biopsy needle. Preliminary in vivo results of this experiment are consistent with the previous clinical trial conducted with Suh et al. The data suggests that there is a unique ESS optical signature which corresponds to benign and malignant thyroid tissue. This data from ESS carries the potential to reduce the amount of diagnostic surgeries done for the most common endocrine malignancy, thyroid cancer. This could potentially save thousands of dollars on thyroid surgery, as well as the life-long dependence on thyroid hormones, and increase the quality of life for patients found to have a thyroid nodule.

The data collected thus far in the study is encouraging. The goal of this research is to create an improved diagnosis for thyroid cancer. We hope that this research will provide the technology necessary to create a single needle biopsy that will form a better diagnosis for patients with thyroid nodules, and provide a better operation for those who have thyroid cancer, while avoiding operations for those who don’t.
JOURNAL ABBREVIATIONS

Ann Surg Oncol. Annals of Surgical Oncology
Arch. Oral Biol. Archives of Oral Biology
CA Cancer J Clin. CA: A Cancer Journal for Clinicians
Diagn. Cytopathol. Diagnostic Cytopathology
Endocr. Rev. Endocrine Reviews
Gastrointest. Endosc. Gastrointestinal Endoscopy
Indian J Urol. Indian Journal of Urology
JAMA Journal of the American Medical Association
JUM Journal of Ultrasound in Medicine
Oral Oncol. Oral Oncology
Ultrasound Med. Biol. Ultrasound in Medicine and Biology
Ultrasound Q. Ultrasound Quarterly
References


VITA

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Year of Birth: 1989

EDUCATION
Boston University, Boston, Massachusetts
• Bachelor of Science: Biomedical Engineering

May 2011

WORK EXPERIENCE
Whitaker Cardiovascular Institute, Lab Manager, BUSM, Boston, MA
• Worked with multiple animal models on projects relating to Biomolecular imaging
• Responsible for operating and maintaining an 11.7 Tesla MRI for BUSM’s Biomedical Imaging Core
• Appointed leadership position training and guiding new students in the techniques and principles of MRI

May 2008 – September 2011

PROJECTS
Spectroscopy Integrated Biopsy Syringe to Detect Thyroid Cancer, Boston Medical Center
September 2012 - Present
• Designed and built an integrated elastic scattering spectroscopy biopsy syringe for use in vivo
• Analyzed data and found trends in spectra corresponding to malignant tissue
• Presented work at numerous conferences around the country in both poster and oral presentations

Transcranial Ultrasound Hemorrhage Detection, BWH, Harvard Medical School
August 2010 – August 2011
• Performed statistical study classifying and characterizing imaging positions on human calvaria
• Constructed a brain phantom that mimics brain tissue in all ultrasound properties with a 2% tolerance
• Worked on an interdisciplinary team to create a program that eliminates secondary backscatter from bone

Optimizing Health Capacity in Resource-Limited Settings, Boston University
October 2010 – May 2011
• Reformatted structural and systematic designs of a hospital in Livingstone, Zambia
• Used a database of maintenance records to establish mean time between failures
• Established a student-run group to generate innovative thoughts on generating, maintaining and sustaining medical devices in a third world country

Magnetization Transfer Contrast in Atherosclerotic Plaque, Boston Medical Center
July 2009 – July 2010
• Studied 4 features of plaque vulnerability that can be identified through MRI
• Performed ex-vivo and in-vivo MRI scans of atherosclerotic plaque and referenced to histology
• Created automated program to perform quantitative T1 and T2 pixel by pixel analysis of plaque

PUBLICATIONS

Circulation (Pending) – Secondary Author
2013

J. Cardiovascular Magn. Reson. (Pending) – Secondary Author
2013

Plos One – Secondary Author
2013

J. Cardiovascular Magn. Reson. - Secondary Author
2012

Ann. Otol Rhinol Laryngol. - Secondary Author
2011

AWARDS

Professional Development Award
2013

Ging S. Lee Community Service Memorial Award
May 2011

Dean’s List 3 of 8 semesters
2010 – 2011

VOLUNTEER EXPERIENCE

Somerville Auxiliary Police, Somerville, Massachusetts
Auxiliary Sergeant, certified as a first responder
Supervise the city of Somerville’s initiative for community policing
January 2011 – Present

Best Buddies, Boston, Massachusetts
Regularly assist with the development of interpersonal skills for people with social disabilities
2007-2011

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<table>
<thead>
<tr>
<th>EXTRACURRICULAR ACTIVITIES</th>
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<tr>
<td>*Graduate Teaching Assistant for Medical Biotechnology</td>
<td>2013</td>
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<tr>
<td>*Peer Mentor</td>
<td>2012-2013</td>
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<td>*Reserve/Intermittent Police Academy</td>
<td>2012-2013</td>
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<td>*Dean's Host</td>
<td>2010-2011</td>
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<td>*Synapse (Undergraduate Student Journal of BU)</td>
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<td>*Alternative Spring Break, HIV Awareness - Atlanta, Georgia</td>
<td>2011</td>
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<tr>
<td>*Alternative Spring Break, Environmental Awareness - Horse Cave, Kentucky</td>
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