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Detailed Terms
Multiscale Imaging of Human Thyroid Pathologies using Integrated Optical Coherence Tomography (OCT) and Optical Coherence Microscopy (OCM)

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ABSTRACT

We evaluate the feasibility of optical coherence tomography (OCT) and optical coherence microscopy (OCM) for imaging of benign and malignant thyroid lesions ex vivo using intrinsic optical contrast. Thirty four thyroid gland specimens were imaged from 17 patients, covering a spectrum of pathology, ranging from normal thyroid to neoplasia and benign disease. The integrated OCT and OCM imaging system allows seamlessly switching between low and high magnifications, in a way similar to traditional microscopy. Good correspondence was observed between optical images and histological sections. The results provide a basis for interpretation of future OCT and OCM images of the thyroid tissues and suggest the possibility of future in vivo evaluation of thyroid pathology.

Keywords: Optical coherence tomography (OCT); Optical coherence microscopy (OCM); Thyroid; Pathology; Cancer.

INTRODUCTION

Thyroid cancer is the most common malignancy of the endocrine system [1]. Approximately 37,200 new cases and 1,630 thyroid cancer deaths are expected in the United States in 2009 [2]. Various methods are used for the detection of and screening of thyroid nodules for malignancy. These include clinical examination, various imaging methods, and ultrasound (US) guided fine needle aspiration (FNA). Thyroid cancer commonly presents as a cold (inactive) nodule on radio-isotope scanning. Up to 40% of adults have a thyroid nodule detected by either palpation or ultrasound.[3-6]. Imaging techniques that can aid in the differentiation between benign and malignant thyroid nodules which may require surgery, are of great interest. However, present imaging methods, including scintigraphy, ultrasound, CT, and MRI, have only limited utility in the routine diagnostic assessment of thyroid nodules [7-9].

Optical coherence tomography (OCT) is a promising technique for real-time, high resolution imaging of tissue morphology [10]. Optical coherence microscopy (OCM) is an extension of OCT, which combines coherence gated detection with confocal microscopy to achieve cellular resolution imaging in the en face plane [11-15]. By enhancing rejection of multiple scattered light, OCM can achieve better image contrast and greater imaging depth [16] with lower numerical aperture [12] compared with confocal microscopy. Integrated 3D-OCT and OCM has the additional advantage of enabling investigation of tissue structure at the architectural and cellular scale. Three dimensional OCT data sets enable cross-sectional and en face
projection imaging, providing large field of view, while OCM provides high magnification enabling cellular imaging. Few studies using integrated OCT and OCM imaging have been performed, largely due to the lack of advanced OCM instrumentation. In this study, we explore the feasibility of using integrated 3D-OCT and cellular resolution OCM imaging for multiscale assessment of human thyroid pathologies ex vivo in a pathology laboratory setting. These results have been recently accepted for publication in the Journal of Biomedical Optics [17].

METHODS

The study protocol was approved by the institutional review boards at the Beth Israel Deaconess Medical Center (BIDMC) and the Massachusetts Institute of Technology (MIT). Informed consent was waived. Freshly excised thyroid specimens were selected based on the presence of pathology upon gross examination. Normal and pathologic tissues were collected from each specimen without interfering with routine pathologic workup. Fresh tissue (typically measured 1 x 1 x 0.5 cm$^3$) from surgical specimens that remained following processing for pathologic examination was collected for imaging and placed in RPMI medium 1640 (Invitrogen, Carlsbad, CA) within 1 hour after excision. Imaging was performed within ~2-6 hours of excision. In total, 34 thyroid gland specimens were imaged from 17 patients (median age, 45 years; 12 females and 5 males). Ten benign and 13 malignant thyroid specimens were imaged. The specimens with benign diagnosis include goiters (n = 4), Hashimoto’s thyroiditis (n = 3) and follicular adenoma (n = 3). The specimens with malignant diagnosis include papillary carcinoma, classic type (n = 6), papillary carcinoma, follicular variant (n = 6) and medullary carcinoma of the thyroid (n = 1). Eleven matched normal thyroid specimens were obtained from total thyroidectomy specimens and evaluated as controls.

A portable, prototype imaging system integrating 3D-OCT and OCM was employed for the study. A detail description of the system design can be found in [18]. The system uses a compact, spectrally broadened, femtosecond Nd:Glass laser light source, which provides >200 nm bandwidth centered at 1060 nm. The output from the laser was split equally into the OCT and OCM subsystems. The OCT subsystem had a <4 μm axial resolution and 14 μm transverse resolution. The axial resolution corresponds to optical image slices thinner than traditional histological sections. A pair of high speed scanning galvonometers enables the beam to be scanned in two dimensions, allowing 640 cross-sectional OCT images to be acquired with 1344 x 1000 (transverse x axial) pixels at 1 frame/second. This results in a three dimensional data set covering a volume of 3 x 1.5 x 1.3 mm$^3$ (X x Y x Z). The OCT signal was demodulated and logarithmically compressed using an analog circuit before analog to digital conversion. The OCM subsystem shares the same sample arm optics as the OCT subunit, except for the objective lens (40x, Zeiss Achromat), which was turret mounted to allow rapid interchange between high (OCM) and low (OCT) magnifications. The aperture of the objective is not fully filled and the resulting confocal parameter is ~30 μm. The transverse image resolution for OCM was ~2 μm. A separate reference arm utilizes dispersion compensating optics and enables an axial resolution of ~4 μm in tissue. The penetration depth of the OCT and OCM system depends on the scattering properties of the sample and over 500 μm and 300 μm imaging depth (respectively) was obtained in human thyroid tissues. A high speed, broadband electro-optic phase modulator was used, enabling rapid image acquisition with raster scanning and demodulation over a 400 x 400 μm$^2$ field (500 x 750 pixels) at 2 frames/second. A detection sensitivity of -98 dB was achieved with ~10 mW of incident power.

A thin cover slip was gently placed on the specimen to create a flat surface and reduce optical aberration. The light pressure applied to the specimen is not expected to influence tissue morphology and histological comparison with OCT/OCM imaging. 3D-OCT images were first acquired. En face OCM data was then collected within the same imaging area to ensure good co-registration. 3D-OCM dataset was also acquired on some specimens by scanning the sample stage in the axial direction at 5 μm/s. After imaging, a photo of the gross specimen was taken before the specimen was marked with black and red ink spots on the imaging surface to indicate orientation. The specimen was formalin fixed and sent for standard histological processing. Sections were cut in en face planes to allow co-registration to both en face OCT and OCM images.
Surfaces of the 2D-OCT cross-sectional images were detected and flattened in post-processing to allow en face image planes to be viewed at constant depth. En face slices of OCT images (3 x 1.5 mm²) were reconstructed from the 3D data sets by averaging over 10 um intervals in the axial direction to reduce speckle noise (Figure 1). The en face OCM data was processed by digital demodulation, pixel re-sampling, spatial filtering (3x3 triangular kernel) and square-root compression of the signal. The OCT and OCM images are contrast adjusted and displayed with an inverse grayscale color-map, where black represents increased reflectivity. In this retrospective study, the entire en face OCT and OCM database and histology slides were first reviewed, and representative normal and pathologic specimens were selected for further evaluation. The registration procedure works well to identify the region of tissue imaged for comparison to histology. However, direct one-to-one registration of image planes to histology remain challenging because the exact orientation of the histological section is difficult to control. At the same time, the generation of volumetric OCT and OCM data provides more comprehensive image information than individual histological sections. Selection was based upon several factors, including the degree to which the images match histology, and the degree to which identified image features accurately represented the larger data set. Representative photomicrographs of histological sections were then made with a best effort attempt to provide comparison with the en face OCT and OCM images. The features used for comparison included, the size and shapes of follicles, papillae, patterns of stromal tissue, calcifications, vascular features, and cellular distribution. This protocol ensured that comparable features were compared on OCT/OCM images and histological sections.

RESULTS

Figures 2 to 4 present examples of data from this study. Representative images from a large spectrum of diseases can be found in reference [17]. En face slices of OCT images (3 x 1.5 mm²) were reconstructed from the 3D data sets by averaging over 10 um intervals in the axial direction to reduce speckle. Figure 2 shows an example where the en face OCT image was at the interface between tumor and normal tissue (Figure 2A). The tumor on the left represents classic type papillary carcinoma, which is the most common type of thyroid cancer in the U.S.(75 to 80%) [19]. The adjacent normal thyroid tissue on the right is separated from the tumor by a dense fibrous capsule. Detailed papillary structures and normal follicles can be seen in the OCM image in Figure 2B and C respectively. Normal thyroid is present as well organized, round or oval thyroid follicles, ranging from 50 to 200 um in diameter in the en face OCT and OCM images. A follicle lined by a single layer of epithelium can be clearly seen in the OCM image of normal thyroid (Figure 2C). In contrast, normal follicles are absent in papillary carcinoma (Figure 2B) and the thyroid is replaced by complex papillary structures. The corresponding histology (Figure 2D to F) matches well with OCT and OCM images.
Figure 2. *En face* OCT (A) shows the tumor interface with normal thyroid tissue in a case of classic type papillary carcinoma. The tumor on the left side is clearly distinguished as densely packed papillary structures (P), separated from the adjacent normal thyroid follicles (F) on the right side by a dense fibrous capsule. Details of papillary structure and normal normal follicles are shown in the OCM images (B, C). (D-F), corresponding H&E slides (4x and 20x respectively). Scale bars, 500μm in (A, D) and 100μm in (B, C, E and F).

Figures 3. A representative case of the follicular variant of papillary carcinoma. *En face* OCT (A) showed a homogeneous micro-follicular (MF) pattern, where the details can be seen under OCM (B). The size of the micro-follicles is approximately 30-50 μm, consistent with the H&E histology in (C, D, 4x and 20x respectively). Scale bars, 500μm in (A, C) and 100μm in (B, D).
Figure 3 is an example of the follicular variant of papillary carcinoma. The nodular thyroid shows a homogenous micro-follicular pattern (Figure 3A and C). Details of the micro-follicles can be seen in the OCM image. The size of the micro-follicles is ~ 30-50 μm, consistent with histology. Although the resolution of OCM is not at the level for cytologic diagnosis of this disease, the clear observation of the microfollicular pattern provides valuable information suggesting the follicular variant of papillary carcinoma (in addition to a microfollicular lesion representing follicular adenoma/carcinoma), which is currently not available in any of the in-vivo imaging modality used in clinical practice.

Figure 4 shows images from medullary carcinoma. Medullary carcinoma is a less common malignant tumor in the thyroid, representing about 3-5% of thyroid carcinomas [19]. The malignant cells are derived from parafollicular cells, or C cells, that normally secrete calcitonin. In medullary carcinoma, normal thyroid follicles are absent. Sheets and nests of tumor cells are surrounded by dense fibrosis which can be clearly seen from the en face OCT image in Figure 4. Details of the tumor nests can be visualized in the OCM image, matching the corresponding histology. Medullary carcinoma can have a variable histomorphologic appearance, so more examples will be sought in the future to better document this spectrum.

![Image](image-url)

**Figures 4.** Sheets and nests of tumor cells (T) are surrounded by fibrous bands (FI) in medullary carcinoma observed with en face OCT (A) and OCM (B) obtained about 50μm below the tissue surface. (C) and (D), corresponding H&E slides (4x and 20x respectively). Scale bars, 500μm in (A, C) and 100μm in (B, D).

**DISCUSSION**

Over the past three decades, a 2.4-fold increase in thyroid cancer incidence was observed in the United States [20]. This was mainly due to an increased use of ultrasound for thyroid screening, which permits detection of nodules as small as 2-3 mm. Several ultrasound features have been associated with an increased risk of thyroid cancer, including presence of calcifications, hypoechogenicity, irregular margins, solid composition, nodule shape, and intranodule vascularity [19]. However, diagnostic accuracy of these criteria for malignancy is dependent on tumor size [21]. Furthermore, considerable overlap between benign and malignant...
characteristics observed with ultrasound has been reported [22, 23], and sensitivity and specificity of malignant nodule differentiation are variable [19, 21, 24-28]. Current guidelines for management of thyroid nodules detected by ultrasound suggest performing FNA on nodules larger than 1 cm to make the diagnosis of a benign or malignant nodule [9, 19]. However, the sensitivity and specificity of thyroid FNA varies [29].

The OCT and OCM technologies presented in the current study have the potential to be a useful complementary technique for the evaluation of thyroid nodules. The axial and transverse resolutions of OCT and OCM are 1-2 orders of magnitude finer than the state-of-the-art ultrasound technology. The integrated OCT and OCM system allows seamlessly switching between low and high magnifications, in a way similar to traditional microscopy. The ability to visualize tissue morphology at multiple scales is very important for pathologists to differentiate clinical relevant features. As a result, characteristic architectural and cellular features from normal thyroid and benign and malignant thyroid diseases were successfully visualized at multiple resolution scales in excised specimens, without exogenous contrast agents or histological processing.

The ability of OCT and OCM to assess follicle shape and delineate growth patterns of thyroid tissues is valuable. The shapes of normal follicles are round to oval. Lesions containing macrofollicles are more likely to be benign, whereas nodules composed predominantly of microfollicles are more likely to be neoplastic. Features visualized in malignant diseases, such as the absence of normal follicles, and the presence of complex papillae, microfollicles, sheet/nests of tumors, are approaching resolution at the cellular level. These intrinsic features form an image base that can be used to differentiate normal and benign nodules from malignancy using OCT and OCM. One limitation in the current study is the relatively small sample size, which prevents us from determining the sensitivity and specificity for assessment of thyroid malignancy. Prospective studies with a larger sample size and blinded image interpretation will be required to establish the clinical utility of OCT and OCM for thyroid neoplasia assessment.

CONCLUSION

In the present study, we evaluate integrated OCT and OCM to assess benign and malignant thyroid tissue ex vivo in freshly excised human thyroid specimens. Images of normal and pathologic tissue were correlated with histologic sections in order to recognize which histomorphologic features could be visualized using integrated OCT and OCM imaging. The results provide a basis for interpretation of future OCT and OCM images of the thyroid tissues and suggest the possibility of future in vivo evaluation of thyroid pathology.

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