Ex vivo Imaging of Human Pathologies with Integrated Optical Coherence Tomography (OCT) and Optical Coherence Microscopy (OCM)

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ABSTRACT

We report an imaging study using integrated 3D-OCT and OCM for the assessment of various human thyroid and breast pathologies. The 3D-OCT data sets enable en face projection imaging, which provides large field of view with uniform focus and signal level, while OCM provides high magnification that enables cellular resolution imaging. We have demonstrated that the integrated 3D-OCT and OCM technology provide a substantial improvement over standard OCT for the visualization of tissue pathology and can serve as a useful imaging tool in the clinical settings.

Keywords: Optical Coherence Tomography, Optical Coherence Microscopy, Human Pathology, Breast Cancer, Thyroid Cancer

1. INTRODUCTION

Early diagnosis is the key to effective therapy and long-term cancer survival. To date, excisional biopsy and histology is the gold standard for disease diagnosis, but can have high false negative rates due to sampling errors. Optical coherence tomography (OCT) is a promising technique for real-time, high resolution imaging of tissue morphology1. Ultrahigh-resolution OCT can achieve 1-2 µm axial resolution in tissue, but is limited in transverse resolution due to the low numerical aperture (NA) focusing used to maintain a sufficient depth of field over the range of the cross-sectional image2. The relatively low lateral resolution achievable with cross-sectional OCT is generally insufficient for imaging of cellular features and therefore limits the utility of OCT in applications requiring cellular level diagnostics. To extend the imaging power of optical coherence tomography to very high transverse resolution, a technology known as optical coherence microscopy (OCM) was developed, which combines optical coherence tomography with confocal microscopy in order to achieve cellular level resolution3-7. OCM can also achieve better image contrast and greater imaging depths8 with lower numerical aperture4 compared to confocal microscopy.

Approximately 182,460 new cases of invasive breast cancer are expected to occur among women in the United States in 2008, accounting for 26% of all cancer cases in women. In the same time period, 40,480 deaths are expected from breast cancer, making it the leading cause of cancer deaths in women after lung cancer9. On the other hand, thyroid cancer is the most common malignancy of the endocrine system10. Approximately 37,340 new cases and 1,590 deaths of thyroid cancer are expected to occur in the United States in 2008. Although these are challenging problems because of the complexity of pathology, breast and...
thyroid cancers are important model systems for visualization cancers characterized by alterations ductal and glandular architecture, epithelial structure, and cellular morphology. OCT and OCM can visualize architectural and cellular morphology in situ and in real time using hand held probes as well as imaging needles which permit imaging inside solid tissue.

The pathology lab imaging studies enable the acquisition of comprehensive 3D-OCT and OCM data sets as well as a more controlled comparison between imaging and histology than possible in vivo. It also provides access to large numbers of specimens which would not be available outside the clinic. Integrated 3D-OCT and OCM has the additional advantage of enabling investigation of tissue structure at the architectural and cellular scale: the three dimensional OCT data sets enable en face projection imaging, which provides large field of view with uniform focus and signal level, while OCM provides high magnification that enables cellular resolution imaging. OCT alone has been employed to image human pathological tissues, such as gastrointestinal tract11-14, breast tissues15-17, urological tissue18,19, and human thyroid20, etc.. However, image resolution was limited to over 10μm. Limited studies using the integrated OCT and OCM techniques have been performed, largely due to the lack of advanced OCM technique. Only very recently, our group has demonstrated the feasibility of imaging human GI tract and thyroid ex vivo using the integrated OCT and OCM method21, 22.

In the present study, we evaluate the feasibility of using integrated OCT and OCM methods for pathological assessment of various human pathologies ex vivo in freshly excised human tissues. Images of normal and pathologic tissue specimens from breast and thyroid were correlated with histology in order to understand the features that could be visualized using the integrated OCT and OCM technique. The results provide a basis for interpretation of future OCT and OCM images of the breast and thyroid tissues and suggest the possibility of using real time optical methods as clinical diagnostic tools.

2. METHODS

2.1. Integrated OCT and OCM system

A portable time-domain prototype imaging system integrating 3D-OCT and OCM was employed for the study. The detail description of the system design can be found in23. The system incorporates a compact spectrally broadened femtosecond Nd:Glass laser, which provides > 200 nm bandwidth centered at 1060 nm with over 100 mW fiber coupled power. The output from the laser was split equally into the OCT and OCM subunits. The OCT subunit was similar to the system used in a previous in vitro imaging study13. Fourteen microns transverse resolution and less than 4 μm axial resolution, which corresponds to optical image slices thinner than traditional histologic sections, was obtained in tissue. A pair of high speed linear scanning galvometers enable the beam to be scanned in two dimensions, allowing 650 cross-sectional OCT images to be acquired with 1344 x 1000 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 (X x Y x Z). The OCT signal was demodulated and logarithmically compressed using an analog circuit before analog to digital conversion.

The OCM subunit shares the same sample arm optics as the OCT subunit, except for the objective lens (40x, Zeiss Achromplan), which was turret mounted to allow rapid interchange between high (OCM) and low (OCT) magnifications. This allows a transverse image resolution of less than 2 μm. A separate reference arm utilizes dispersion compensating optics and enables nearly transform limited axial resolution of less than 4 μm in tissue. The fast galvometers and a high speed, broadband electro-optic phase modulator were used, enabling rapid raster scanning and demodulation over a 400 x 400 μm field (500 x 750 pixels) at 2 frames/second. Detection sensitivity of -98 dB was achieved with ~10 mW of incident power to the specimens. A custom autofocusing technique was used to ensure the matching of the coherence gate and the confocal gate positions.
in the OCM images \(^2\). The integrated OCT and OCM system was compact and can be fit into two portable instrument carts.

### 2.2. Imaging Protocols

The imaging protocol was approved by the institutional review boards at the Beth Israel Deaconess Medical Center (BIDMC) and the Massachusetts Institute of Technology (MIT). Freshly excised human thyroid (43) and breast specimens were selected based on the presence of pathology upon gross examination following prompt arrival to the pathology laboratory. Tissue (typically measured 1 x 1 x 0.5 cm) from surgical specimens deemed unnecessary for diagnosis by supervising pathologists was immediate transported to imaging facility in RPMI medium 1640 (Invitrogen, Carlsbad, CA) within 1 hour. Imaging was performed within \(\sim\)2-6 hours of excision. After imaging, the sample was formalin fixed before sent for histologic processing. Sections were cut in \textit{en face} planes to allow co-registration to both the \textit{en face} OCT and OCM images. Slides were stained with hematoxylin and eosin (H & E) and histologic pictures were digitally recorded using a standard microscope.

### 3. RESULTS

Figure 1 and 2 shows representative images from the breast specimens. Characteristic patterns of normal human breast are shown in Figure 1. An \textit{en face} projection at about 200 um below the tissue surface was constructed from 3D-OCT images, demonstrating uniform focus and signal level across a large field of view.

![Figure 1](image.jpg)

Figure 1. Characteristic pattern of normal breast. (A) \textit{En face} projection of breast tissue from 3D-OCT images shows fibrostic tissues (FI), blood vessels (V) and breast glands (G) clearly. Detailed structure of breast glands can be observed through the OCM image (B). (C, D) Corresponding histology. Scale bars, 500um in (A, C) and 100um in (B, D).
Figure 2. Characteristic pattern of a breast fibroadenoma. (A) *En face* projection over 20 um breast tissue from 3D-OCT images. (C) Corresponding histology enables identification of fibroptic tissues (FI) from the *en face* OCT projection and OCM (B). (C, D) Corresponding histology. Scale bars, 500um in (A, C) and 100um in (B, D).

Figure 3. A. *En face* OCT shows the tumor-normal junction of a classic type papillary carcinoma. The tumor on the left hand side were clearly distinguished as densely packed papillae (P), separated from the adjacent normal thyroid follicles (F) on the right by dense fiber (FI) capsules. Details of papillae and normal follicles are shown in the OCM images (B, C). (D-F), corresponding H&E slides. Scale bars, 500um in (A, D) and 100um in (B, C, E and F).
Figure 4. En face OCT (A) and OCM (B) show follicles (F) with significantly varying sizes, which is a representative feature of follicular adenoma. (C, D), corresponding H&E slides. Scale bars, 500μm in (A, C) and 100μm in (B, D).

(~1.5 mm x 3 mm). Fibrostic tissues (FI), blood vessels (V) and breast glands (G) can be clearly identified. Detailed structure of breast glands can be observed through the magnification of OCM imaging (Figure 1B), corresponding well with H&E histology (Figure 1C, D).

Figure 2 illustrates the characteristic pattern of a breast fibroadenoma. En face projection of 3D-OCT images of the specimen was constructed (Figure 2A), demonstrating uniform focus and signal level across a large field of view (~1.5 mm x 3 mm). Fibrostic tissues (FI) can be clearly identified from the en face OCT projection and OCM (Figure 2B) and correspond well to the H&E histology (Figure 2C, D).

Figures 3 and 4 are representative examples from human thyroid specimens. Figure 3 shows OCT and OCM images obtained at the tumor-normal junction. Round or oval thyroid follicles (F), ranging from 50 to 500μm in diameter, were seen well organized on the right side of the en face OCT image (Figure 3A). The follicle lined by a single layer of epithelium with colloid can be clearly seen in the OCM image (Figure 3B). Normal thyroid is separated from the tumor on the left by dense fiber (FI) capsules. The tumor is consistent with classic type papillary carcinoma (P), which is the most common type of thyroid cancer (65 to 80% in USA)\textsuperscript{24}. Normal follicles are absent in the tumor regions. Instead, the thyroid was replaced by complex papillae. Detailed papillae structure can be appreciated from the OCM image shown in Figure 3C. The corresponding histology slides (Figure 3D-F) demonstrated good matches to the OCT and OCM images.

Follicular adenoma is a benign encapsulated tumor with follicular differentiation throughout the nodule. Figure 4 shows a representative case. As can be seen from the OCT and OCM images, the follicle size varies significantly, ranging from 40 μm to 800 μm in diameter. This observation was consistent with histology and was also confirmed by images from other specimens.
4. DISCUSSIONS

Compared to conventional cross-sectional OCT images, the en face OCT images reconstructed from the 3D-OCT dataset have demonstrated several advantages. For example, the en face OCT images shown in this study were obtained at the same depth from the surface. This allows uniform focus and signal level to be achieved, preserving image contrast over the entire field of view. The en face OCT also greatly facilitates co-registration with histology. The integration of the OCT and OCM imaging technologies makes great analogue to a standard microscope: a lower magnification (OCT) was used to evaluate tissue architectural features, while a higher magnification (OCM) was used to evaluate cellular details. This approach was demonstrated effective in the current study and can be readily accepted by the pathological communities.

In a conventional confocal microscope, both the transverse resolution and the axial resolution are determined by the numerical aperture (NA) of the objective. However, the resolution in the axial dimension increases at a much slower rate compared to the transverse dimension as the NA increases. In order to achieve an axial resolution of < 5 μm, which is equivalent to the thickness of a conventional histologic section, a typical NA of 0.7 – 1.0 would be utilized. OCM, on the other hand, combines optical coherence and confocal detection. Therefore, the transverse and the axial resolution are effectively de-coupled: the transverse resolution is still determined by the NA of the objective, while the axial resolution is determined by the combination of objective NA and the bandwidth of the light source. For example, an effective NA of ~ 0.3 – 0.4 provides a high transverse resolution of ~2 μm in the current system, although this allows a confocal gate of ~ 20 μm in the axial dimension. The addition of the coherence detection helps to improve the axial resolution to <4 μm, which was mainly attributed to the broad bandwidth of the light source. Since the development of miniaturized high NA objective lenses remains challenging, the use of coherence gated detection to relax the NA requirements should facilitate the development of needle based miniature probes to guide FNA at cellular resolution with OCM.

5. CONCLUSIONS

In conclusion, we have used an integrated OCT and OCM system to image a spectrum of breast and thyroid pathologies ex vivo. Our preliminary results identified architectural and cellular features previously obtained only by conventional biopsy. The results of these studies demonstrated that the integrated 3D-OCT and OCM technology can be used reliably in the clinical setting and provide a substantial improvement over standard OCT for the visualization of tissue pathology. With continued development of microscanning probes, needle-based and endoscopic OCT and OCM promise to be important tools for applications in early cancer detection.

6. ACKNOWLEDGEMENTS

This work was supported by the NIH grants R01-CA75289-12 (JGF and JLC), Air Force Office of Scientific Research contract FA9550-07-1-0014 (JGF) and Medical Free Electron Laser Program contract FA9550-07-1-0101 (JGF). THT acknowledges support from the Taiwan Merit Scholarship from the National Science Council of Taiwan. The authors acknowledge useful discussions with Desmond C. Adler.
REFERENCES