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Award Number: DAMD17-01-1-0156

TITLE: Optical Coherence Tomography of the Breast: A Feasibility Study

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REPORT DATE: October 2003

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 074-0188

1. AGENCY USE ONLY (Leave blank)	2. REPORT DATE	3. REPORT TYPE AND DATES COVERED
	October 2003	Annual (1 Oct 02-30 Sep 03)

4. TITLE AND SUBTITLE
Optical Coherence Tomography of the Breast: A Feasibility Study

5. FUNDING NUMBERS
DAMD17-01-1-0156

6. AUTHOR(S)
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8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSORING / MONITORING AGENCY REPORT NUMBER

11. SUPPLEMENTARY NOTES

12a. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for Public Release; Distribution Unlimited

12b. DISTRIBUTION CODE

13. ABSTRACT (Maximum 200 Words)

Optical Coherence Tomography (OCT) is an emerging high-resolution imaging technology that can perform high resolution, real-time cross-sectional imaging of tissue. OCT can be used as a type of "optical biopsy" to perform minimally-invasive imaging up to a depth of 2-3 mm with transverse resolutions as high as 10 um in commercially available systems. OCT uses near-infrared light which can be used in fiber optic devices such as catheter probes and imaging needles. This novel imaging technology has the potential to improve cancer detection and diagnosis. The aim of this study was to determine the feasibility of applying OCT imaging to normal and pathologic human breast tissue, as well as other human tissues. OCT was used to image samples of breast tissue from core biopsy and surgical specimens. Architectural changes such as stromal hyperplasia and fat necrosis were detected with OCT. Normal lactiferous ducts were visible. However, normal glandular and ductal structure as well as pre-malignant and neoplastic epithelial changes did not have sufficient contrast to be consistently visible. We are currently improving OCT resolution and applying spectroscopic and microscopy techniques to enhance tissue contrast. These improvements should allow better visualization of microstructural features in normal and pathologic breast tissue. Studies were performed imaging other tissues with better defined architectural morphology including thyroid and lower GI tissues, and good correlation of OCT images and histology was obtained.

14. SUBJECT TERMS
Novel imaging of the breast

15. NUMBER OF PAGES
11

16. PRICE CODE

17. SECURITY CLASSIFICATION OF REPORT	18. SECURITY CLASSIFICATION OF THIS PAGE	19. SECURITY CLASSIFICATION OF ABSTRACT	20. LIMITATION OF ABSTRACT
Unclassified	Unclassified	Unclassified	Unlimited

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INTRODUCTION

Optical coherence tomography (OCT) is an emerging imaging modality which can generate micron-resolution, cross sectional images of tissue pathology *in situ* and in real time [1-4]. OCT imaging has a resolution higher than any clinically available imaging modality and imaging can be performed in real time under endoscopic guidance. Optical coherence tomography has the advantage of higher resolution than current clinical ultrasound without requiring tissue contact or imaging through a transducing medium. OCT can also be incorporated in a wide variety of endoscopic and laparoscopic imaging devices [5]. OCT has the particular advantage of enabling visualization of microscopic mucosal and submucosal architectural features which are not visible using clinically available imaging methods.

OCT imaging is analogous to ultrasound B mode imaging, except that imaging is performed using light rather than sound. A schematic of the OCT system used is shown in Figure 1. High resolution, cross sectional images are generated of the internal structure in tissue by directing and scanning a beam of light onto the tissue and measuring the echo time delay of backscattered or back-reflected light. OCT images are two dimensional data sets that are typically displayed as a grey scale or false color image and represents the optical scattering or reflections in a cross sectional plane (Figure 2).

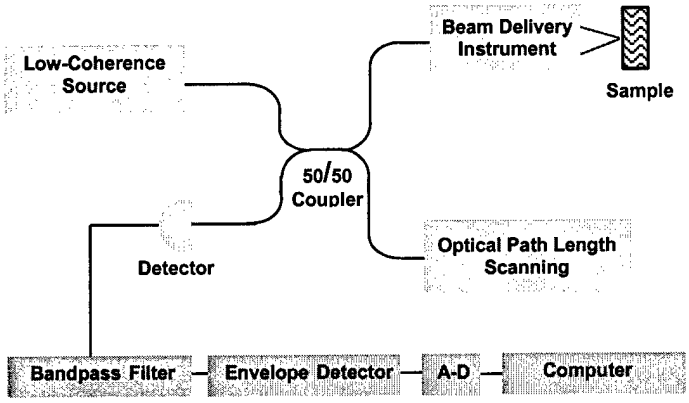

Figure 1: Schematic of the fiber-optic implementation of an OCT system. High-resolution measurements of the echo time delay and intensity of backscattered light are performed using a correlation technique where low coherence light from the tissue is interfered with light that travels a known reference path length delay in a fiber-optic interferometer.

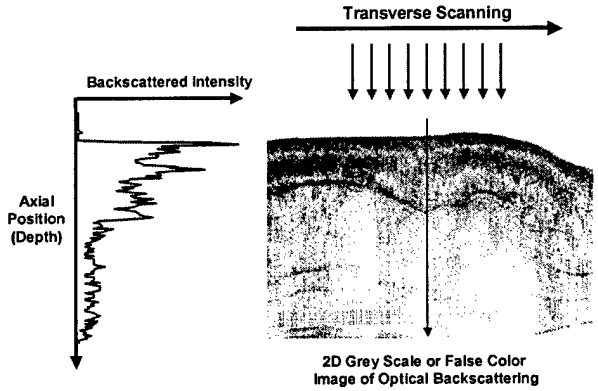

Figure 2: Cross-sectional images are created by scanning the light beam across the tissue sample while recording the axial reflectance profile at each transverse position. The resulting two-dimensional array is displayed as a logarithmic gray scale or false color image representing a cross section of the tissue structure.

BODY

In the past grant period, the group at Massachusetts Institute of Technology (MIT) has made several improvements to OCT imaging technology. Advances in solid-state laser and nonlinear fiber development have enabled the construction of portable, compact, broadband light sources which can be used for ultrahigh resolution imaging. Improving image resolution allows smaller structures in tissues, such as glands and cells, to be identified. Broadband sources have enabled the resolution to be improved by up to 10x, significantly improving image quality [6]. In addition to improved resolution, use of broad bandwidth sources also allows the available wavelengths to be used for spectroscopy. Since tissues scatter and absorb different wavelengths of light to different degrees, spectroscopic OCT may have the ability to differentiate tissue components, such as water or oxygenated versus deoxygenated hemoglobin in blood. This technique of "spectroscopic staining" could be used as an analogy to staining in histopathology, enhancing image contrast and providing better differentiation of tissue pathologies. Spectroscopic OCT would also enable functional imaging of tissue on the micron scale. This research program is leveraged by ongoing research programs from the Air Force Office of Scientific Research, Medical Free Electron Laser Program, contract F49620-01-1-0186 "OCT Imaging & Delivery for Surgical Guidance" and National Institute of Health, NIH-5-R01-CA75289-06 "Optical Biopsy using Optical Coherence Tomography" at M.I.T. which synergistically support portions of the technology development in this program.

Development of Portable Ultrahigh Resolution Systems

The group at MIT has recently developed two new promising ultrahigh resolution imaging systems. One of these systems uses a source operating at ~1.3 um wavelength, based on the Cr:Forsterite femtosecond laser. Tissue is relatively transparent to light in this wavelength because absorption from the primary tissue chromophores (blood, melanin, and water) is relatively low and scattering is much less than for shorter wavelengths. Femtosecond pulses from the laser can be coupled into a dispersion-shifted nonlinear fiber to broaden their spectrum to greater than 200 nm, corresponding to better than 4 um axial image resolution. This system is portable and can produce images at 2-4 frames per second, allowing imaging of specimens in real time. This light source has an output power of ~40 mW, which is ideal for high speed imaging [7].

A second system recently developed by the group at MIT works at a center wavelength of ~1.0 um. Operating in the 1.0 um wavelength region allows higher resolution to be obtained for the same optical bandwidth, at the expense of slightly decreased tissue penetration. This may be ideal for detecting early stage cancers which arise in the relatively thin surface epithelial layers of tissue. This system uses a commercially-available femtosecond modelocked Nd:Glass laser in conjunction with a high numerical aperture fiber. Image resolutions of ~5 um were achieved at speeds of several frames per second. This commercially available Nd:Glass light source has the advantage of being compact, turnkey, and reliable [8].

Cellular Level Imaging using Optical Coherence Microscopy (OCM)

While development of broadband laser technology has enabled axial resolutions of 1-3 um, the transverse resolution of traditional OCT has been limited to 15-25 um, too large to resolve individual cells and subcellular features which are important for many applications. Transverse resolution in OCT has recently been dramatically enhanced by applying another imaging technique called Optical Coherence Microscopy (OCM), which is closely related to OCT [9].

OCM is similar to OCT, but instead of generating cross-sectional images, it generates en face images similar to those of microscopy. Imaging en face has the advantage that the entire image is kept within the

focal plane of the focusing lens. OCM is similar to confocal microscopy except that it uses the coherence gating methods of OCT to enable better rejection of backscattered light from outside the focal plane of the sample. Because coherence gating is extremely effective at reducing scattered light, it can perform imaging at greater depths than possible using confocal microscopy alone. Using coherence gating also relaxes the requirement of using large high numerical aperture focusing lenses, simplifying objective lens designs and promising to enable the development of small probes that can be used in laparoscopes or endoscopes for imaging cells inside the human body. This improved transverse resolution also enables imaging of cells in vivo, which is relevant for many applications including early cancer detection.

The combination of the high sensitivity and coherence-gated detection of OCT with confocal microscopy fundamentally extends the capabilities of OCT for imaging at the cellular level. We are planning to apply this technique to imaging normal and pathologic human breast tissue, as well as other human tissues.

KEY RESEARCH ACCOMPLISHMENTS

Pathology Laboratory Imaging

In the past grant period we have completed a preliminary imaging study at the pathology laboratory using the new 1.3 um portable high speed imaging system developed at MIT. Ultrahigh resolution OCT was used to image a survey of normal, inflammatory, and neoplastic tissues in vitro in the pathology laboratory at BIDMC. Imaging in the pathology laboratory setting allowed imaging of fresh surgical tissue specimens, which eliminates artifacts introduced by tissue autolysis or fixation and most closely approximates tissue contrast that would be expected for imaging *in vivo*. Imaging in the pathology laboratory also enables control of imaging parameters and accurate registration of OCT images with histology.

A novel compact solid-state chromium-forsterite laser operating at a center wavelength of 1260 nm with a bandwidth of 130 nm was used for this study. Using this laser light source, it was possible to achieve an axial resolution of ~4.5 um in tissue, a factor of 2 to 3 times higher than conventional OCT systems. The transverse image resolution was determined by the focused spot size of the optical beam, which is related to the numerical aperture of the focusing lens and the wavelength of the light source as in conventional microscopy. The transverse spot size diameter for the OCT system used in this study was approximately 18 um. Imaging was performed using a surgical imaging probe (measuring 1 cm in diameter and 15 cm in length) mounted on a precision stage to provide controlled displacements of the light beam orientation and position. Imaging was performed at 2-4 frames per second, enabling real time visualization and adjustment of the imaging plane. Imaging was performed without contact with the tissue to prevent alterations in scattering and brightness due to pressure of the probe on the tissue.

Imaging was performed on freshly excised surgical specimens available in the pathology laboratory. Specimens from the breast, thyroid, gastrointestinal tract, as well as other tissues were imaged. Following optical biopsy, oriented tissue samples were processed in the histology laboratory and 5 um sections (8-10 levels) were obtained and stained with Hematoxylin & Eosin. Digital OCT images & histology sections were then compared and correlated to determine the ability of OCT to visualize tissue architecture and cellular morphology and the ability to distinguish normal & benign from malignant tissue. Results from the breast, thyroid, and lower gastrointestinal tract are reported here. Data from other tissues is currently being analyzed.

Ultrahigh Resolution Imaging of the Breast

OCT was used to image samples of breast tissue from core biopsy and masectomy specimens taken for in-situ or invasive carcinoma. More than twenty samples (1.0 x 0.5 cm each) were taken from benign & lesional breast tissue of each specimen and placed in 10% buffered formalin and/or phosphate buffered saline. Imaging was performed both in the laboratory at MIT and in the pathology laboratory using a recently developed, portable ultrahigh resolution system. Imaging in the pathology laboratory of surgical specimens was performed on freshly excised tissue to eliminate possible fixation artifacts. Imaging was performed using bench top and real-time ultrahigh-resolution imaging systems, operating at 800 or 1300-nm wavelengths respectively, with an axial image resolution of 1.5 to 5.5 μm (corresponding to 1-4 μm in tissue) and a transverse image resolution of 5 to 18 μm. Image data was stored digitally and reviewed. Following imaging, oriented tissue samples were processed in the histology laboratory and 5 μm serial sections (8-10 levels) were obtained, in the same plane that the probe had scanned, and stained with Hematoxylin & Eosin. OCT images & histology sections were then compared and correlated to determine the feasibility of using OCT for distinguishing normal & benign from malignant breast tissue.

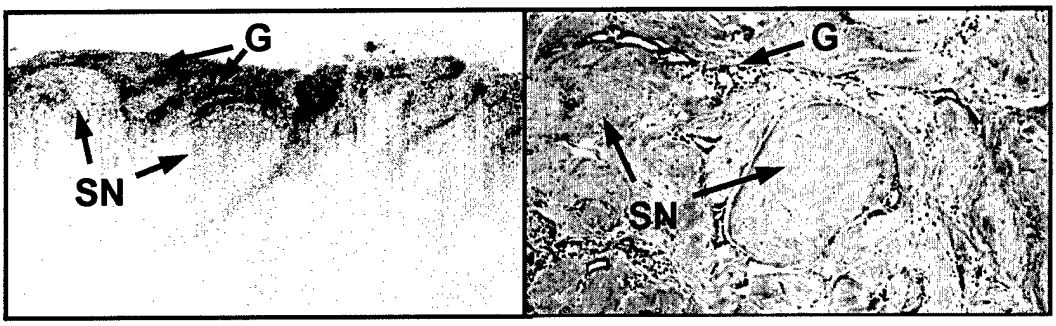

Figure 3. OCT image of fibroadenoma. Stromal nodules (SN) and intervening glands (G) are apparent both the OCT image (left) and histology (right).

Architectural changes such as stromal hyperplasia and fat necrosis were detected with OCT. Normal lactiferous ducts were visible, as well as mucinous stroma in one case of fibroadenoma. Fibroadenoma architecture, including stromal nodules and intervening glands, were visible on some images (Figure 3). However, normal glandular and smaller ductal structures as well as pre-malignant and neoplastic epithelial changes did not have sufficient contrast to be consistently visible. Adipose tissue provided the best contrast from normal stromal non-adipose tissue, though epithelial structures were more difficult to resolve. Cases with ductal carcinoma *in situ* and invasive ductal carcinoma with desmoplasia were difficult to resolve. Until the tissue imaging contrast can be improved, it is not feasible to use OCT for distinguishing normal from potentially malignant lesions of the breast.

Ultrahigh Resolution Imaging of the Thyroid

Current diagnostic imaging modalities of the thyroid gland cannot reliably distinguish benign from malignant lesions, primarily because of their inability to visualize microscopic structure. A high-resolution imaging technique capable of examining thyroid tissue architectural morphology, previously possible only by conventional biopsy, could help in the evaluation of the thyroid gland.

The feasibility of optical coherence tomography for imaging thyroid tissue was explored *ex vivo* on the human thyroid gland. High-resolution optical coherence tomography was performed in real time at 2-4 frames on three post-mortem and 15 surgically excised thyroid glands containing normal, hyperplastic and neoplastic tissue. Two post-mortem thyroid glands were entirely normal and one gland had an incidental

papillary carcinoma. Seven cases of multinodular goiter were imaged. These goiters were comprised of multiple thyroid nodules ranging in size from 0.3 cm to 3.5 cm, and were admixed with areas of cyst formation, hemorrhage, scarring and dystrophic calcification. Three other specimens with benign solitary nodules measuring up to 1.9 cm in diameter were evaluated. Three adenomas, one (1.7 cm) with a predominant microfollicular growth pattern and two (1.5 cm and 3.1 cm) with Hürthle cell changes, were scanned. There were also two surgical specimens containing papillary carcinoma (both follicular variants), measuring up to 2.2 cm. Lymphocytic thyroiditis was noted in two thyroid glands. All specimens were serially sectioned. Grossly normal and scarred areas as well as nodular and cystic lesions were imaged. Optical coherence tomography images acquired were compared with those obtained using standard histopathologic methods.

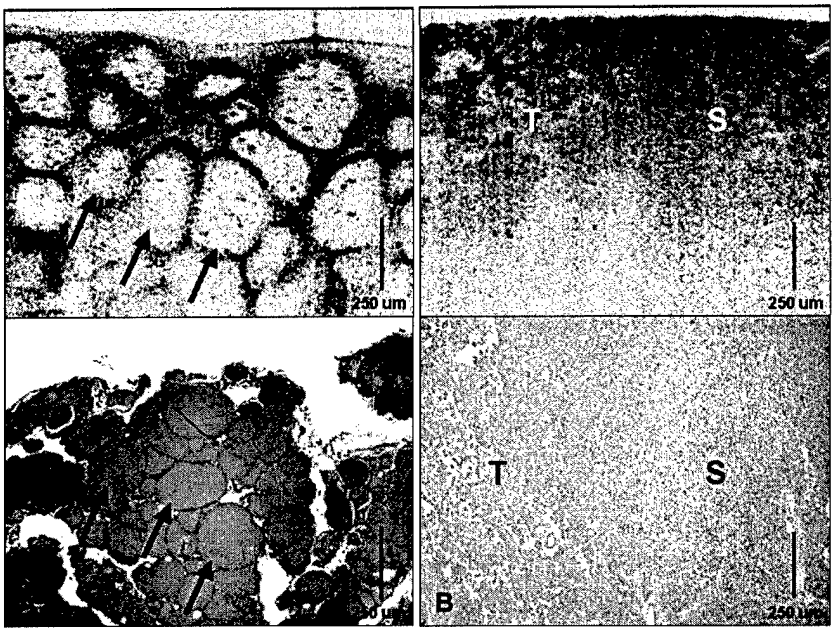

Figure 4. A. Normal thyroid tissue showing (arrows) multiple colloid-filled follicles. B. Adenoma with areas showing trabecular (T) and solid (S) growth patterns. (OCT top; histology bottom).

In this study it was demonstrated for the first time that high-resolution cross-sectional imaging of thyroid tissue using OCT is feasible. The microstructure of the normal thyroid gland and adenoma, including colloid-filled follicles and growth patterns, were clearly identified (Figure 4). Optical coherence tomography images of degenerative, hyperplastic, adenomatous and malignant change within the thyroid gland were shown to correlate well with corresponding histopathologic findings. OCT images povided reliable and clinically useful information about the microstructure of normal, benign and malignant thyroid tissue that until now was only attainable by means of conventional histopathologic examination.

Further clinical evidence is still required to determine if OCT would be able to distinguish thyroid malignancies from benign thyroid lesions. These preliminary results suggest that OCT is a promising diagnostic tool in the evaluation of patient's with thyroid disease, with the potential to better select patients with a high risk of having a malignant lesion. Since the prognosis of thyroid cancer is partially dependent on its size at surgery, earlier beneficial detection of cancer may be possible using OCT for screening. These results have been submitted to the journal *Head and Neck* [10].

Ultrahigh Resolution Imaging of the Lower Gastrointestinal Tract

The purpose of this study was to investigate new ultrahigh resolution OCT technology, which has image resolutions a factor of 2 to 3 times finer than standard endoscopic OCT, for imaging pathology of the intestinal tract in vitro. Optical coherence tomography has the advantage of higher resolution than any currently available clinical imaging technique. Imaging in the pathology laboratory setting also enables control of imaging parameters and accurate registration of OCT images with histology which is difficult to achieve for in vivo endoscopic OCT imaging. OCT also enables visualization of microscopic mucosal and submucosal architectural features which are not visible using clinically available imaging methods. Endoscopic OCT has also been shown to provide complementary information to endoscopic ultrasound for potential applications in staging of endoscopic tumor resection [11]. Because of these advantages, there has been significant interest in using OCT for improving current diagnostic techniques for detecting early stage disease. The results of this study provide a basis for interpreting future endoscopic OCT studies using ultrahigh resolution imaging.

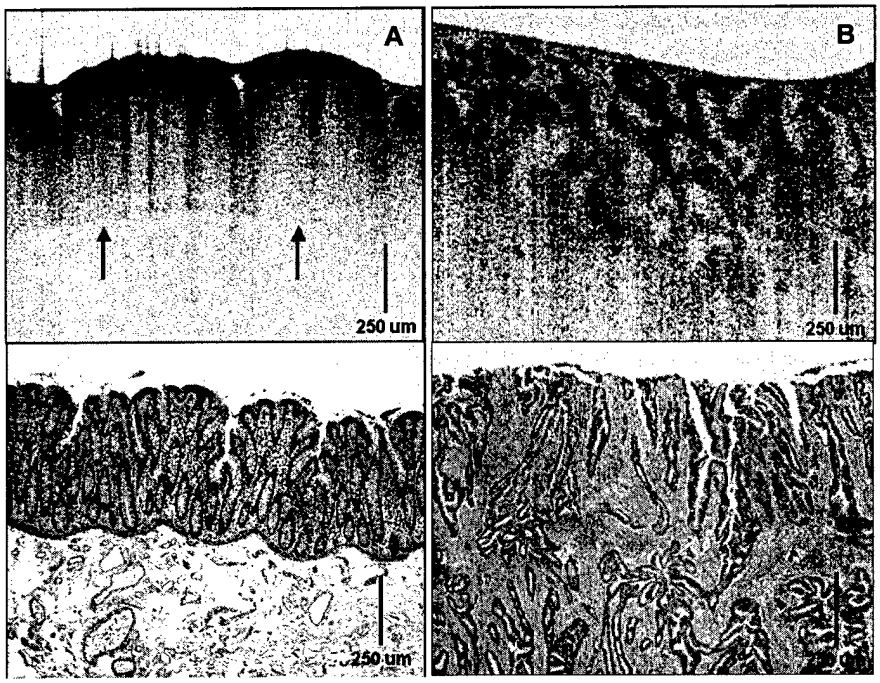

Figure 5. A. OCT image of normal colon. Mucosa is clearly delineated from underlying submucosa by a defined high scattering band corresponding to the muscularis mucosa (arrows). The submucosa is loose and less optically scattering B. OCT image of well differentiated adenocarcinoma. Highly irregular invasive glands are visible in a desmoplastic stroma. No clear boundary between mucosa and submucosa is evident. (OCT top; histology bottom).

Ultrahigh resolution OCT imaging was performed in the pathology laboratory on freshly excised specimens from the lower gastrointestinal tract using axial image resolution of 4.5 um with real time imaging at 2-4 frames per second. Normal and diseased intestinal tissues from surgical resections were imaged within two hours of excision. A total of 52 areas from 18 cases from adult patients were scanned. Normal intestinal sites imaged included (i) small intestine (n = 8) and (ii) colon (n = 16). Diseased tissues imaged included (i) inflammatory bowel disease (n = 9; ulcerative colitis n = 8; Crohn's disease n = 1), and (ii) neoplasia (n = 6) with one submucosal lipoma, one tubulovillous adenoma involving the duodenum, three sites of invasive carcinoma involving the transverse colon, and one squamous cell carcinoma in the anorectal region. Grossly normal as well as ulcerated and scarred lesions were imaged. The OCT image

planes were carefully registered with microinjections of ink, formalin-fixed, and processed using routine histological methods. OCT images were correlated with corresponding histologic sections.

Ultrahigh resolution OCT demonstrated clear delineation of the mucosa and submucosa, as well as improved visualization of mucosal glands, crypts, villi, and mucosal folds. Normal architectural variation along the intestinal tract could be visualized in OCT images, as well as architectural distortions due to gland irregularity and ulcerations from inflammatory and malignant processes. Benign tumors localized within the submucosa were visible, as well as areas of malignant change invading the submucosa could also be visualized.

This study can be used as a baseline for interpreting future ultrahigh resolution endoscopic OCT studies. The results presented here suggest that ultrahigh resolution endoscopic OCT can be a powerful research and clinical tool for the study of gastrointestinal tract pathologies and an important adjunct to current imaging methods. These results are in preparation for submission [12].

REPORTABLE OUTCOMES: OCT imaging was performed simultaneously on breast, colon/rectum, thyroid, and other surgical specimens as part of this study. These results have been reported in two manuscripts, one accepted to the journal *Head and Neck* and one in preparation for *Gastrointestinal Endoscopy* [10, 12]. A review paper on OCT is being published in *Nature Biotechnology* [13].

CONCLUSIONS: Preliminary imaging studies of the breast are underway, but require improvements in image resolution. Promising results have been obtained in thyroid and GI pathologies. The group at the Massachusetts Institute of Technology is updating the OCT technology to apply spectroscopic and microscopy techniques to improve the resolution and enhance tissue contrast. These improvements should allow better visualization of microstructural features in normal and pathologic tissue. Additional breast tissues will be imaged after the improvements have been completed to determine if changes enable differentiation of normal vs. pathologic tissue.

REFERENCES:

1. Huang, D., et al., *Optical coherence tomography.* Science, 1991. **254**(5035): p. 1178-1181.
2. Fujimoto, J.G., et al., *Optical biopsy and imaging using optical coherence tomography.* Nature Medicine, 1995. 1(9): p. 970-972.
3. Brezinski, M.E., et al., *High-resolution imaging of plaque morphology with optical coherence tomography.* Circulation, 1995. **92**(8): p. 103-103.
4. Fujimoto, J.G., et al., *Optical coherence tomography: an emerging technology for biomedical imaging and optical biopsy.* Neoplasia, 2000. **2**(1-2): p. 9-25.
5. Tearney, G.J., et al., *In vivo endoscopic optical biopsy with optical coherence tomography.* Science, 1997. **276**(5321): p. 2037-9.
6. Drexler, W., et al., *In vivo ultrahigh-resolution optical coherence tomography.* Optics Letters, 1999. **24**(17): p. 1221-1223.
7. Hsiung, P., et al. *Ultrahigh resolution OCT of prostate pathology in the clinic using a portable Cr:forsterite laser.* in *SPIE Photonics West - BIOS.* 2003. San Jose, CA: SPIE Int. Soc. Opt. Eng. Proceedings of Spie the International Society for Optical Engineering.
8. Bourquin, S., et al., *Ultrahigh resolution real time OCT imaging using a compact femtosecond Nd:Glass laser and nonlinear fiber.* submitted to Optics Express.
9. Aguirre, A., et al., *High-resolution optical coherence microscopy for high-speed, in vivo cellular imaging.* accepted to Optics Letters, 2003.
10. Pantanowitz, L., et al., *High-resolution imaging of the thyroid gland using optical coherence tomography.* submitted to Head and Neck.

11. Das, A., et al., *High-resolution endoscopic imaging of the GI tract: a comparative study of optical coherence tomography versus high-frequency catheter probe EUS.* Gastrointest Endosc, 2001. **54**(2): p. 219-24.
12. Hsiung, P., et al., *Ultrahigh resolution imaging of the lower gastrointestinal tract using optical coherence tomography.* in preparation.
13. Fujimoto, J.G., *Optical coherence tomography for ultrahigh resolution in vivo imaging.* Nature Biotechnology, 2003. **Forthcoming**.