Optical Biopsy with Optical Coherence Tomography[a]

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INTRODUCTION

Conventional excisional biopsy, in combination with histologic examination, is a powerful tool for the diagnosis of a wide range of medical disorders. However, in many clinical scenarios, excisional biopsy is not performed either because tissue excision is hazardous or false negative rates are high. Therefore, a clinical need exists for a technology capable of 'optical biopsy,' the imaging of tissue microstructure at or near the level of histopathology without the need for tissue removal. In this work, we describe the use of the micron scale, cross-sectional imaging technology, optical coherence tomography (OCT), for optical biopsy of human bone and testis.

OCT is analogous to ultrasound, using infrared light rather than acoustical waves.[1] OCT uses low-coherence interferometry to generate micron-scale, cross-sectional images of biological tissue.[2,3] OCT performs imaging in biological tissues by directing an optical beam of infrared light onto the tissue and measuring the reflected or backscattered intensity of light from microstructures within tissue as a function of depth. Though penetration is limited to a few millimeters, the resolution of OCT, which is as high as 4 μm, represents an improvement of up to 25 times that of high-frequency ultrasound, magnetic resonance imaging (MRI), or computerized tomography (CT).

Several features of OCT suggest it is attractive for *in vivo* medical diagnostics in addition to its high resolution. First, OCT is fiber-optic based, allowing inexpensive

[a] This research is supported in part by the National Institutes of Health, Contract NIH-9-RO1-EY11289-10 (JGF), the Medical Free Electron Laser Program, Office of Naval Research, Contract N00014-94-1-0717 (JGF), the Air Force Office of Scientific Research, Contract F49620-95-1-0221 (JGF), the Joint Services Electronics Program, Contract DAAH04-95-1-0038 (JGF), the Whittaker Foundation, Contract 96-0205 (MEB), and the National Institutes of Health, Contract NIH-1-R29-HL55686-01A1 (MEB).

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integration with endoscopes and catheters. Second, unlike ultrasound, OCT does not require direct contact with tissue or a transducing medium. Third, OCT systems are compact and portable, a requirement in endoscopy suites or catheterization laboratories. Finally, unlike MRI or CT, OCT can be performed at high speed, allowing large numbers of high-resolution images to be acquired over short periods.

OCT was originally developed to image the transparent tissue of the eye with unprecedented resolution.[1,4] Preliminary clinical studies demonstrate that OCT can noninvasively image structures of the anterior eye and retina with unprecedented resolution. OCT has been especially promising for the diagnosis and monitoring of macular diseases.[1,4–6] Some quantitative informtion can also be obtained from OCT images, it may also provide the first objective clinical assessment of diseases such as glaucoma.

Recent modifications, which include the use of longer wavelengths in the near infrared, have allowed OCT imaging to be performed in nontransparent tissue.[7,8] This has included the identification of tissue pathology in the cardiovascular system, gastrointestinal tract, and skin.[7,9,10] A feasibility has also been suggested for imaging in the urinary tract, nervous system, respiratory tract, and female reproductive tract.[8–11] The development of a high-speed, catheter-based OCT imaging system has allowed *in vivo* imaging to be performed on rabbit respiratory and gastrointestinal tract.[12,13]

In this work, the ability of OCT to perform optical biopsy is demonstrated by performing imaging on *in vitro* postmortem samples of the skeletal system (bone) and the male reproductive tract.

METHODS

Human tissue was obtained within 12 hr of the postmortem examination. Over 60 sites from 5 patients were examined. The samples were stored in 0.9% saline with 0.1% sodium azide at 0°C. Imaging was performed on segments smaller than 8 cm by 8 cm. The position of the OCT beam on the tissue was followed with a visible light guiding beam. The peripheral areas of imaged sections were marked with microinjections of dye. Imaging was performed at room temperature. Following imaging, the speicmens underwent routine histologic processing. Different microstructures were identified by staining with hematoxylin/eosin (H/E) and trichrome blue. Stained histologic sections were compared with OCT images to allow correlations.

The principles that govern OCT imaging have been previously described.[1,7] OCT measures the echo delay time for incident light to be reflected back from different internal structures within tissue in a manner analogous to ultrasound. However, due to the high speeds associated with the propagation, unlike ultrasound the echo delay time cannot be measured electronically. Therefore, a technique known as low-coherence interferometry (fiber optic Michelson interferometer) is used. A schematic of the OCT system is shown in FIGURE 1. OCT imaging is performed when low-coherence infrared light is coupled into an optical fiber. The light is divided evenly by a beamsplitter, half toward the sample and half toward the reference arm. The path length in the reference arm is varied, here using a moving mirror. Light is reflected back from the mirror and from within the tissue. The backreflected beams are recombined in the beamsplitter. The interferometric signal is detected only when the reflections from the sample and reference arms of the interferometer are nearly matched in group delay. The intensity of interference is

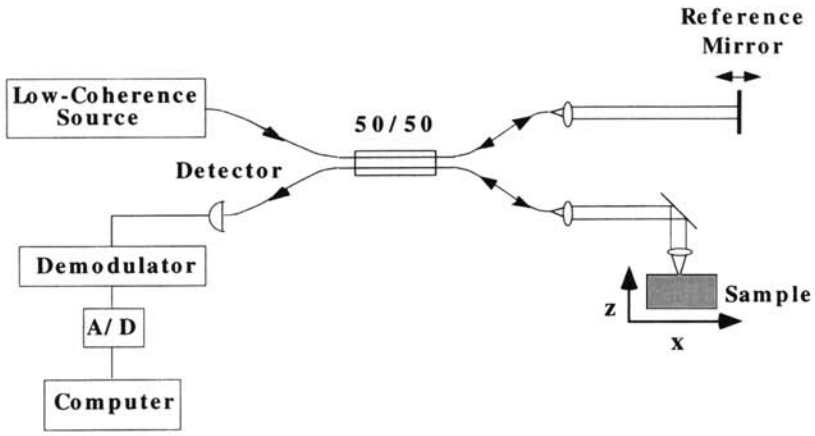

FIGURE 1. Schematic of OCT system.

used to represent the intensity of backreflection and is plotted as a function of depth. The optical beam is scanned across the sample, and sequential axial measurements are taken at different transverse positions to construct two- or three-dimensional data sets.

The axial resolution with OCT is inversely proportional to the bandwidth of the source. A superluminescent diode with a 1300-nm wavelength and a 50-nm bandwidth was used as the light source in this study. The axial resolution was measured to be 16 ± 1 μm from the point spread function off the surface of a mirror. The transverse resolution was determined by a Standard Air Force Resolution Chart to be 30 μm. The signal-to-noise ratio (SNR) was 109 dB, using an intensity of 160 μW at the sample. The SNR was determined by measuring the maximum detected signal when the optical beam is reflected from a mirror divided by the variance of the background noise level of the instrument. Images of backscattering intensity versus distance were displayed in gray scale or false color. The axial dimension of the images correspond to 10 μm/pixel. The acquisition rate ranged from 25–45 sec. However, systems with acquisition rates of 4–8 frames/sec are now available.[13]

RESULTS

The degree of activity of chronic bone disorders, such as osteomyelitis, is difficult to monitor over long periods due in part to the difficulties associated with repeated excisional biopsy.[14] A technology capable of optical biopsy would likely improve the ability of physicians to monitor this relatively common disorder. In FIGURE 2, images of human cortical bone are shown. FIGURE 2A is a cross-sectional image of a human clavicle. The superficial periosteum (P) as well as underlining Haversian systems are sharply differentiated. FIGURE 2B is an image showing the long axis of the bone. The Haversian systems are now seen to run parallel with this axis. The bar represents 500 microns in all images.

Disorders of both the male and female reproductive tract often require microsurgical intervention to restore fertility.[15] Unfortunately, these procedures are often associated with relatively low success rates. A high-resolution imaging technology capable of imaging below the tissue surface could substantially improve the success rates of these procedures. In FIGURE 3A, a cross-sectional image of a testis is shown, which has been transected in a manner similar to procedures used to reverse vasectomies. Individual seminiferous tubules (arrow) are seen. In FIGURE 3B, the width of the tunica albuginea (TA) is identified.

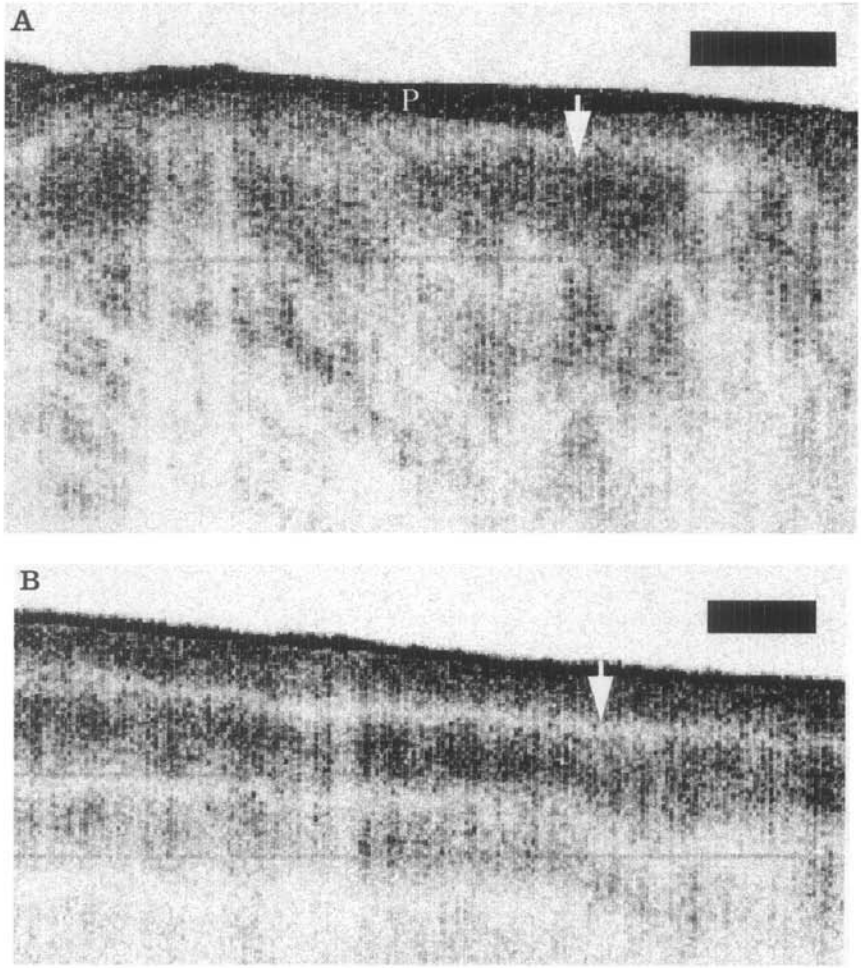

FIGURE 2. Human cortical bone. **(A)** Cross-sectional image of a human clavicle. The superficial periosteum (P) as well as underlining Haversian systems are sharply differentiated (*arrow*). **(B)** Image showing the long axis of the bone. The Haversian systems (*arrow*) are now seen to run parallel with this axis. *Bar* represents 500 microns in all images.

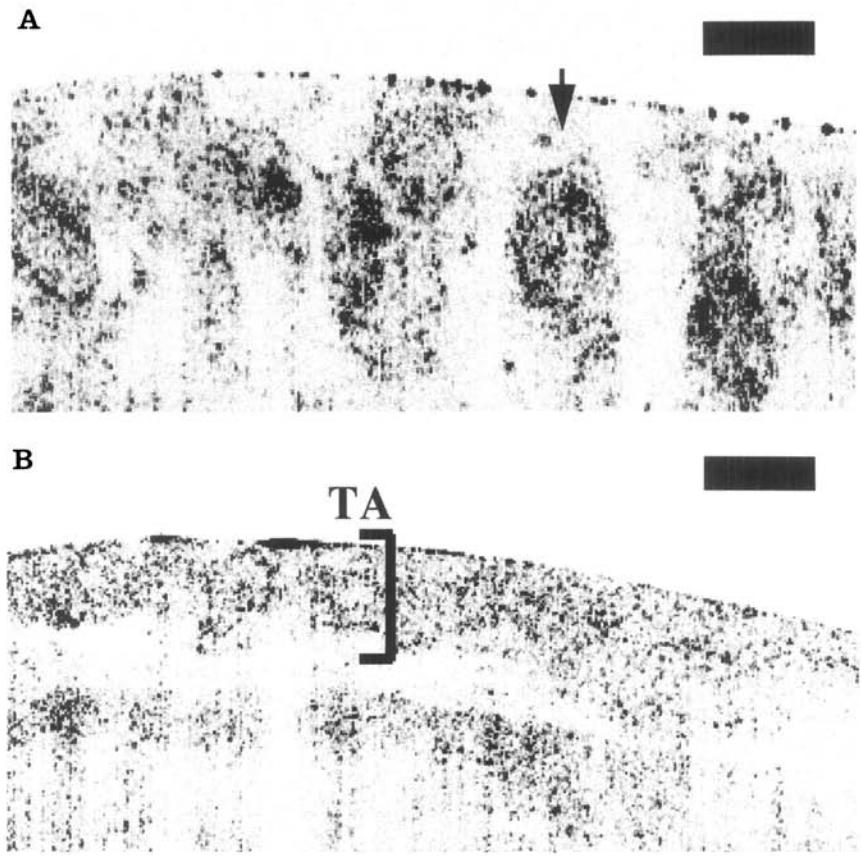

FIGURE 3. Testis. **(A)** Cross-sectional image of a testis that has been transected in a manner similar to procedures used to reverse vasectomies. Individual seminiferous tubules (*arrow*) are seen. In **(B)**, the width of the tunica albuginea (TA) is identified.

DISCUSSION

A technology capable of performing optical biopsy will likely have a large clinical impact in at least three scenarios. The first is situations where excisional biopsy is hazardous or difficult, such as the identification of pathology in the cerebrum or articular surface. The second is circumstances where false negative rates are high due to sampling errors. This would include the screening procedures used in the premalignant states of Barrett's esophagus and inflammatory bowel disease. The third is where high-resolution imaging will likely reduce iatrogenic injury and improve the success rate of surgical and microsurgical procedures.

In this work, optical biopsy was performed with OCT on the male reproductive tract and the musculoskeletal system. Microstructural details, such as the seminiferous tubules and Haversian systems, were delineated at an axial resolution of

16 μm. Effective image penetration ranged from 1–2 mm. A role of OCT for optical biopsy in these organ systems is suggested.

The development of both an OCT imaging catheter/endoscope and a rapid data acquisition system have been major steps toward the ultimate use of OCT for clinical diagnostics. A 2.9 French (1 mm) OCT imaging catheter/endoscope has recently been developed.[12] The catheter contains relatively inexpensive optical components and no transducer within the catheter frame, making it viable for clinical use. A rapid data acquisition system has also been constructed which allows imaging at 4–8 frames/sec.[13] *In vivo* endoscopic imaging has been performed at 10-μm resolution of rabbit esophagus and trachea. Imaging rates near video speed are expected with future developments.

Future modifications, in addition to increased data acquisition rates, will likely improve the performance of OCT. Of particular importance are changes in source wavelength and bandwidth. Both penetration and contrast in OCT images are dependent on the wavelength of the incident source. It has been demonstrated that imaging at 1300 nm allows a dramatic increase in penetration compared with 800 nm.[7] Examination of other wavelengths in the near infrared could yield further improvements in both penetration and contrast. In addition, the resolution of the OCT system is dependent on the bandwidth of the source. Recently, broad bandwidth, short pulse femtosecond laser sources have been shown to yield resolutions in the range of 2–4 μm.[16] Light sources with these broad-bandwidth characteristics have the potential to provide the clinician with cellular-level resolution.

In conclusion, the feasibility of OCT for imaging of the male reproductive tract and bone was demonstrated in this work. OCT represents a promising new technology for optical biopsy of a wide range of medical disorders due to its micron scale resolution, compatibility with catheters/endoscopes, and relatively inexpensive portable design.

SUMMARY

A need exists in medicine for a technology capable of 'optical biopsy,' imaging at or near the resolution of histopathology without the need for excisional biopsy. Optical coherence tomography (OCT) is a recently developed imaging technology that uses infrared light to generate cross-sectional images on a micron scale. In this work, the feasibility of OCT for optical biopsy was confirmed with *in vitro* tissue from the skeletal and male reproductive systems. This work supports the hypothesis that OCT is an attractive technology for *in vivo* optical biopsy.

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