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Optical Biopsy with Optical Coherence Tomography: Feasibility for Surgical Diagnostics[1]

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Background: Optical coherence tomography (OCT) is a recently developed compact technology which uses infrared light to perform cross-sectional imaging on a micrometer scale. Since OCT provides imaging at a resolution comparable to conventional histology and does not require direct contact with the tissue surface, a role in real-time surgical diagnostics represents a logical extension. In this work, we test the feasibility of OCT for surgical diagnostics by demonstrating imaging in tissue relevent to microsurgical intervention, a previously undescribed observation. Materials and methods: Over 50 sites on nervous, reproductive, and microvascular specimens from 10 patients were examined postmortem with OCT. After imaging, tissue was registered with microinjections of dye, under visible light laser guidance, followed by routine histologic processing to confirm the identity of microstructure. Results: The 16 ± 1 μm resolution allowed subsurface microstructure to be identified at unprecedented resolution. Structures identified included fascicles of peripheral nerves, the internal elastic membrane of microvessels, and the granular layer of the cerebellum. Conclusions: The ability of OCT to provide micrometer-scale definition of tissue microstructure suggests a role in surgical diagnostics. Future *in vivo* investigations are merited to establish its utility for morbidity reduction associated with surgical intervention.

INTRODUCTION

The surgical discipline, by addressing pathology on smaller scales, now effectively manages disorders which were previously beyond the scope of traditional medical therapeutics. Improved methods for visualizing microanatomy, in addition to technical refinements in tissue manipulation, have been the cornerstone of this advance in microsurgical interventions [1, 2]. Furthermore, the range of disorders amenable to surgical repair will likely expand with the introduction of new imaging technologies which are superior or complimentary to those currently available. These new technologies ideally would provide the surgeon with superior, real-time information about tissue microstructure and yet have a low profile within the operative field. In this work, we suggest the feasibility of using optical coherence tomography to provide high-resolution real-time imaging for surgical diagnostics and guidance.

Optical coherence tomography (OCT) is a recently developed technology which provides micrometer-scale tomographic imaging of biological tissue [3–5]. OCT is analogous to ultrasound B mode imaging, using infrared light rather than sound waves. The intensity of backreflected light from structures within tissue is plotted as a function of depth. Tomographic images are produced by scanning the optical beam across the sample, generating two- and three-dimensional data sets similar to radar. Thus, an OCT image represents a cross-sectional, micrometer-scale picture of the optical reflectance properties of tissue. Though the penetration is limited to a few millimeters, the resolution of this optical technology, which is as high as 4 μm, represents an improvement of over 25× that of high-frequency ultrasound, MRI, or CT [6, 7]. Therefore, OCT performs "optical biopsy," imaging of tissue microstructure at a resolution only previously available with conventional

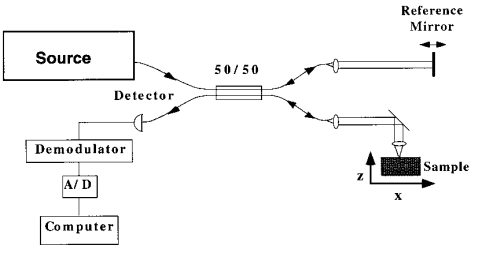

FIG. 1. Schematic of the OCT system.

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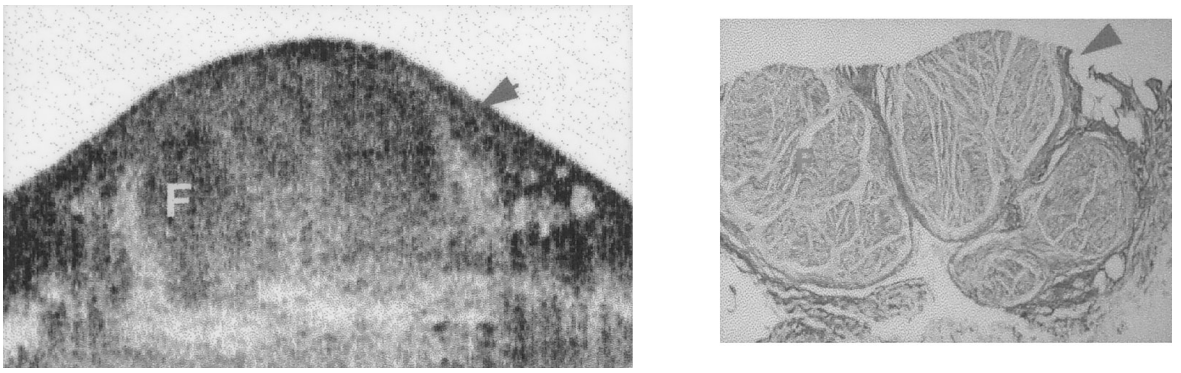

FIG. 2. Peripheral nerve. An OCT image of a peripheral nerve is shown in this image with the corresponding histology. Microstructure within the nerve is clearly delineated including the individual fasicles (F) and epineurium (arrowhead).

histopathology. OCT was introduced to image the transparent tissue of the eye. It is currently under clinical investigation for a wide range of retinal diseases [3, 8, 9]. Recently, imaging has been extended to *in vitro* nontransparent tissue which primarily includes atherosclerotic human aorta [7, 10, 11].

Several features of OCT suggest that it may be well suited to surgical diagnostics in addition to the high resolution. Since OCT is based on fiber-optic technology, no transducer or separate microscope is necessary within the surgical field. Low-profile imaging can be performed by the surgeon through small optical fibers

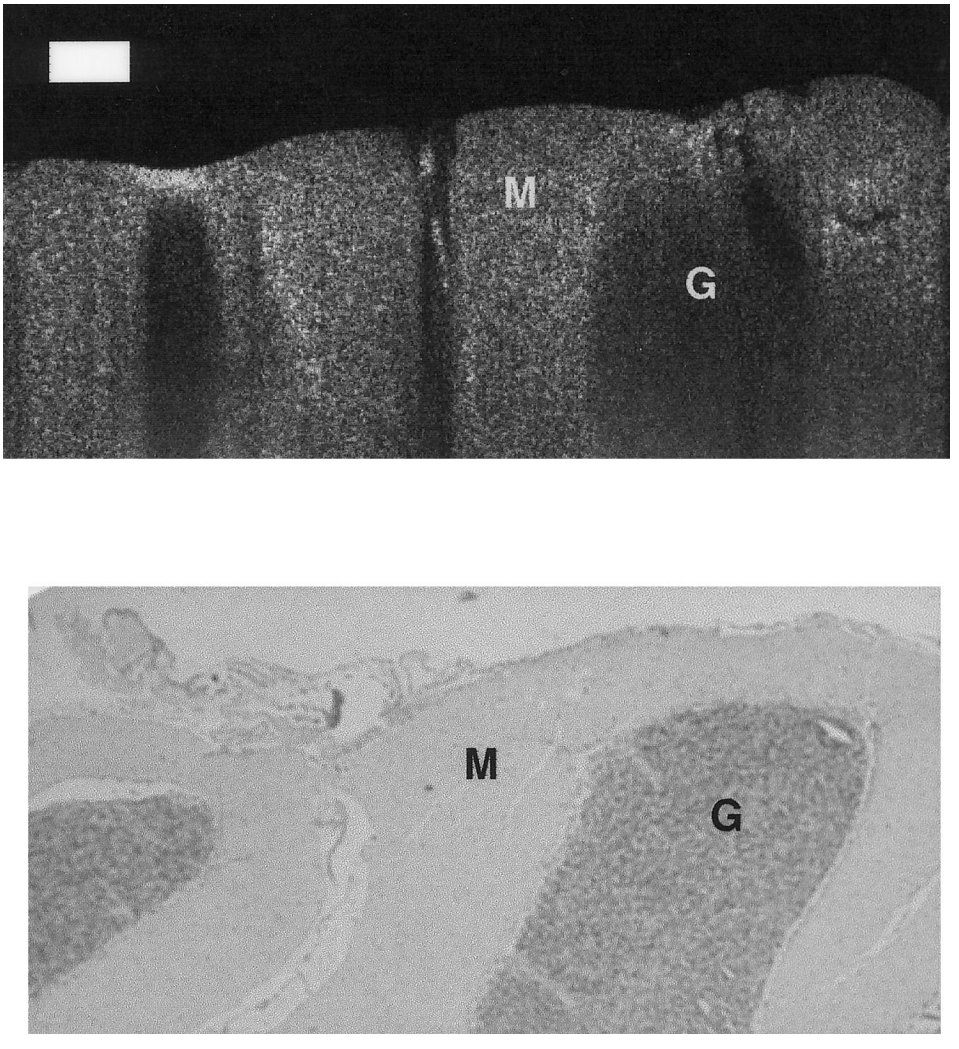

FIG. 3. Cerebellum. An image of a lobule of the cerebellum with the corresponding histology is demonstrated. The molecular (M) and grandular (G) layers are identified. Bar represents 500 μm.

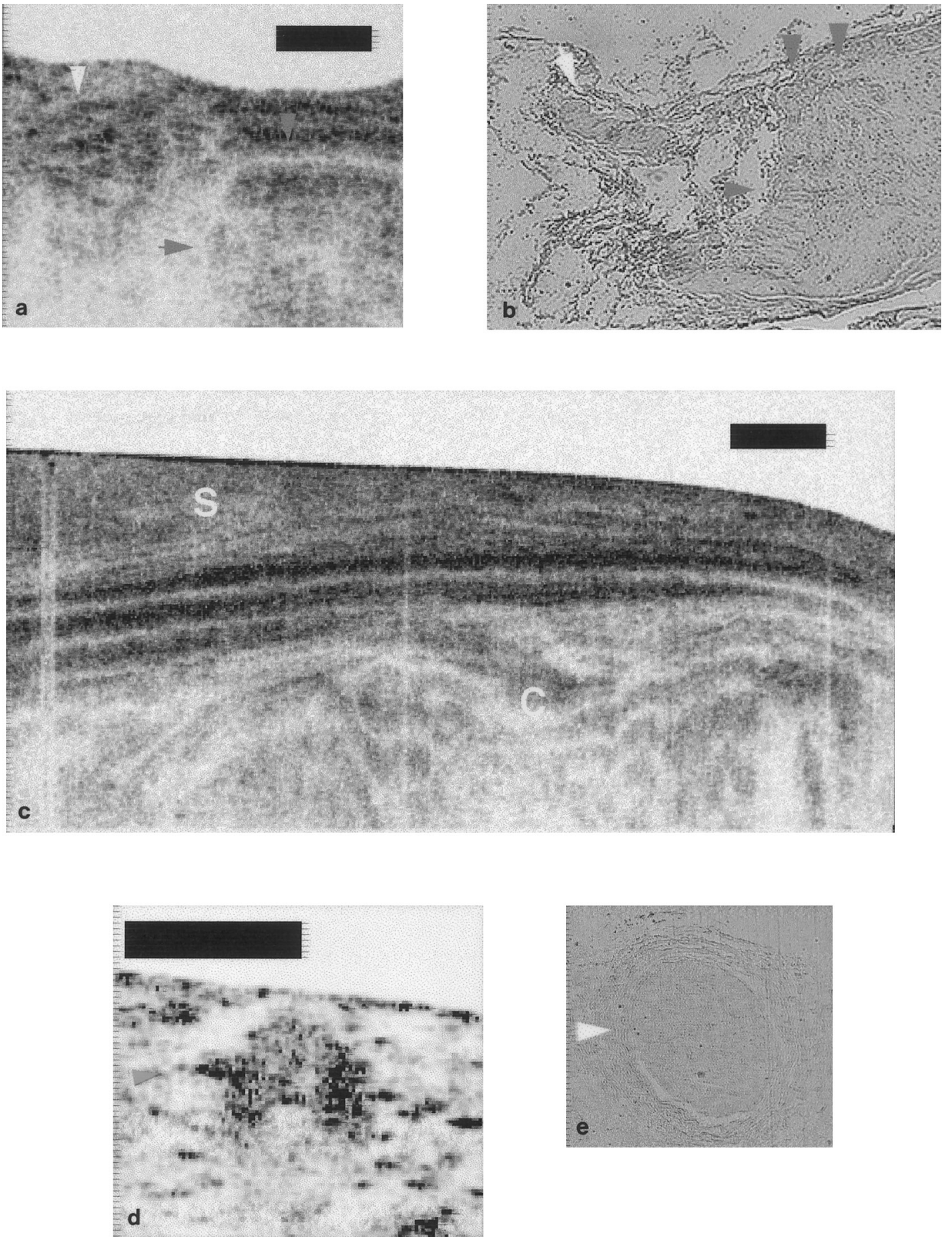

FIG. 4. Unmyelinated nerves. (a) An unmyelinated nerve (left arrowhead) is demonstrated less than 500 μm from an intervertebral disc (right arrowhead). The bar represents 500 μm. (b) The corresponding histology is shown. (c) A larger image of the intervertebral disc is shown with the characteristic fibrocartilage (C) pattern. The overlying supportive tissue is also noted. (d) A small unmyelinated nerve is noted embedded in the periprostate adipose. Bar represents 500 μm. (e) The histology is also shown.

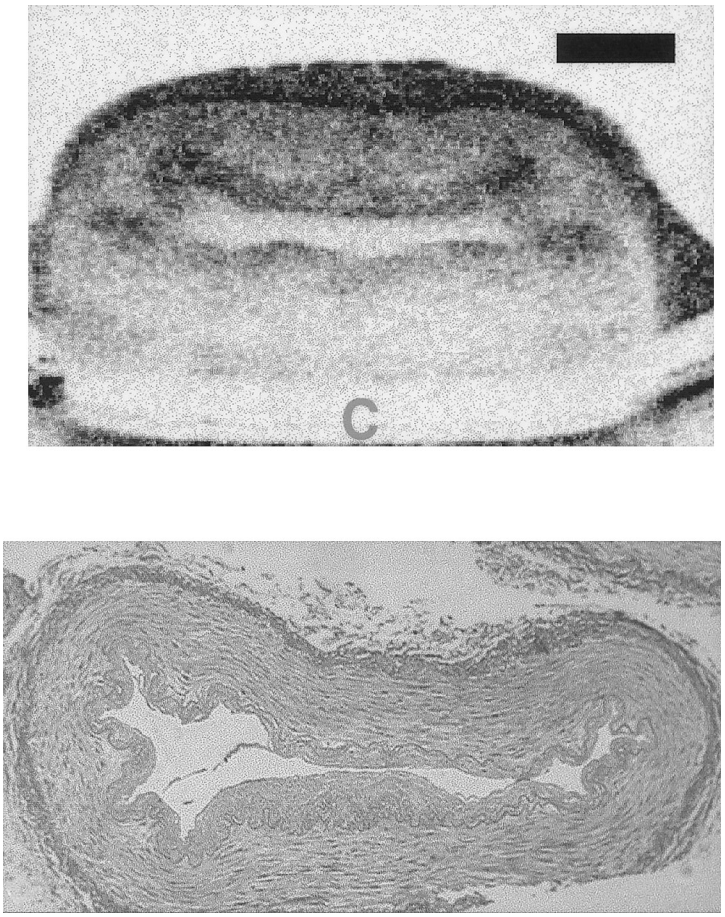

FIG. 5. Small vessel. A branch of the superior mesenteric artery is shown. The intima, media, and adventitia are well differentiated. The coverslip (C) is also imaged through the width of the vessel. Bar represents 500 μm. The corresponding histology has also been included.

attached directly to a scalpel, tissue probe, endoscope, or microscope, providing forward imaging of tissue microstructure. Unlike MRI or CT, OCT is compact and portable (approximately the size of a standard PC), allowing mobility within the tight confines of the operative suite. In addition, in contrast to conventional microscopy and loupes, OCT will provide the surgeon with diagnostic information from below the tissue surface. Finally, unlike ultrasound, OCT imaging can be performed directly through air over distances of a few meters, avoiding the need for direct contact with tissue or the requirement of a transducing medium.

In this work, our hypothesis is that high-resolution imaging can be performed with OCT on *in vitro* tissue of the microvascular, reproductive, and nervous system, an observation which has not been previously described. These tissue types have been chosen because of their surgical relevance. They represent tissue where fine surgical manipulation is frequently required for reconstructive procedures but significant limitations exist with current modalities used for guidance (1). We will therefore suggest a feasibility of OCT for imaging during fine surgical procedures, meriting future *in vivo* investigations.

MATERIALS AND METHODS

Human nervous, reproductive, and vascular tissues were obtained within 6 hr of the postmortem examination. More than 50 sites from 10 patients were examined postmortem with OCT. 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 sample was monitored with a visible light guiding beam. The peripheral areas of imaged sections were marked with microinjections of dye. Imaging was performed with specimens at room temperature. After imaging, the specimens underwent routine histologic processing, being fixed in formalin for 48 hr followed by paraffin embedding. Sections of 5 μm thickness were cut at marked imaging sites, then stained with hematoxylin/eosin (H/E) and trichrome blue to identify different microstructures. Stained histologic sections were semiquantitatively compared with OCT images to provide a better qualitative understanding of the reflectance properties of tissue structures.

The principles which govern OCT imaging have been previously described [3, 7]. The schematic of the OCT device is shown in Fig. 1. OCT measures the delay time for incident light to be reflected back (echo delay time) from different internal structures within tissue. Since the velocity of light is extremely high, the echo delay time cannot be measured electronically as it is for sound. Therefore, OCT measures the echo delay time of light by using a technique known as low coherence interferometry, which utilizes a reference pulse [3, 7]. The resolution of OCT is dependent on the coherence length of the light, which is analogous to the pulse duration. In systems using a mode-locked femtosecond, solid-state laser, resolutions in the range of 4 μm have been achieved [6].

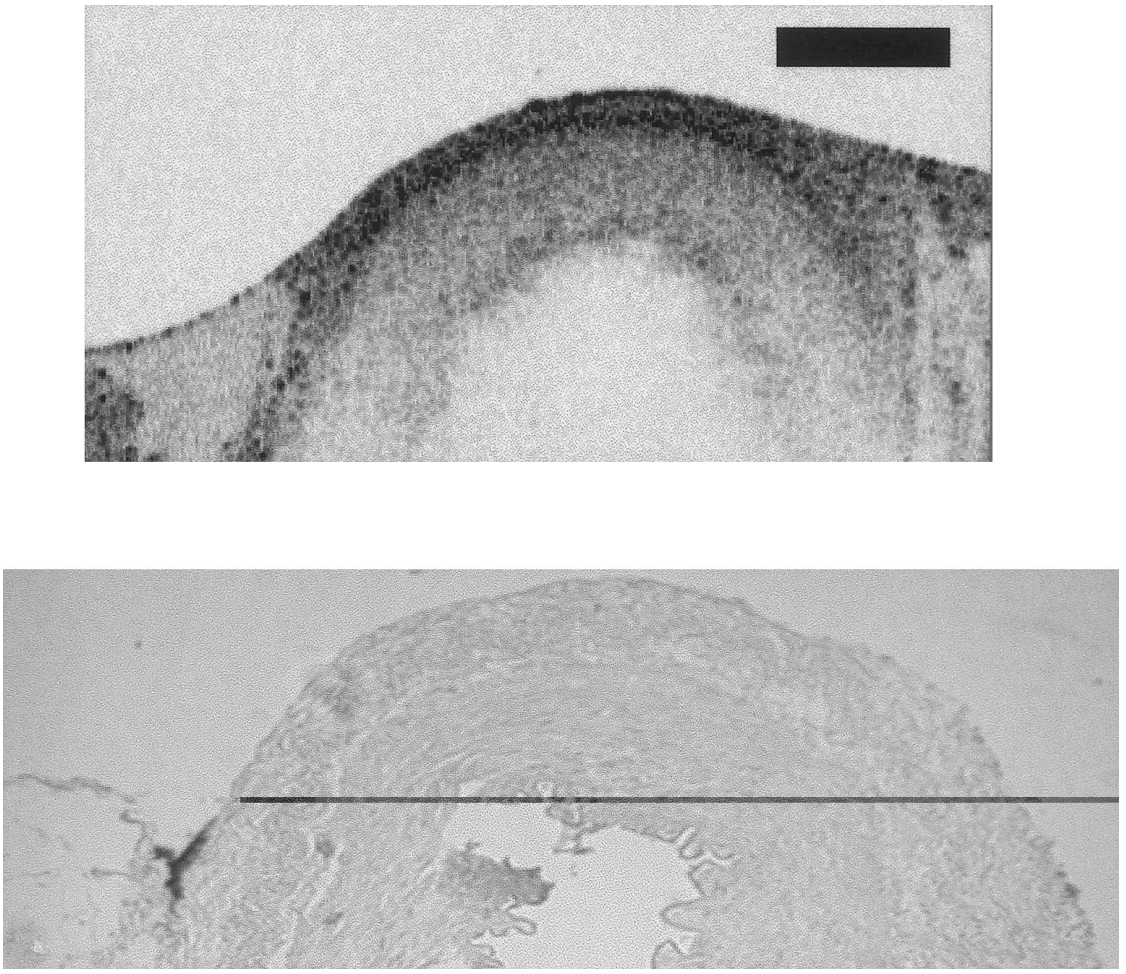

FIG. 6. Small vessel. A branch of the inferior mesenteric artery is shown partially embedded in adipose. Bar represents 500 μm. Corresponding histology has been included.

A 1300-nm superluminescent diode source (bandwidth 50 nm) was used and allows an axial (depth) resolution of 16 $\pm$ 1 μm, determined experimentally by measuring a point spread function off a mirror [7]. The transverse resolution was 30 μm and was determined by the spot size (focusing properties of the system). The signal to noise ratio was 109 dB, using an intensity of 160 μW at the sample. The axial dimensions of the images corresponded to 10 μm/pixel. The acquisition times ranged from 10 to 45 sec depending on the size of the image.

RESULTS

Nervous Tissue

The close proximity of "vulnerable" microstructure within nervous tissue suggests that high-resolution imaging may be beneficial in reducing the morbidity associated with neurosurgical procedures [12]. Figure 2 shows a cross-sectional image of a peripheral nerve. The fascicles (F) are sharply differentiated from the surrounding epineurium (arrowhead). Histology has been provided to confirm tissue identity. The image in Fig. 3 shows a lobule of the cerebellum and was used to represent tissue from the central nervous system. The molecular (M) and granular (G) layers are well differentiated in this image. Therefore, high-resolution imaging is possible of structures within both the central and peripheral nervous systems.

Since injury of small nerves during surgical procedures can result in iatrogenic injury, intraoperative real-time high-resolution imaging may substantially reduce the morbidity associated with tissue dissection of vulnerable regions. An example is the surgical decompression of nerves compromised by displaced intervertebral discs [13]. In Fig. 4a, the OCT image demonstrates a small nerve (approximately 300 μm, left arrowhead) within 500 μm of an intervertebral disc (right arrowhead). A larger image of the intervertebral disc is shown in Fig. 4c, where the characteristic fibrocartilage pattern of cartilage is noted. In Fig. 4d, a small nerve (approximately 300 μm) in the periprostate adipose is shown, with the corresponding histology in Fig. 4e. Iatrogenic damage to these small unmyelinated periprostatic nerves can result in impotence or dysfunction of micturition [14]. A similar role could be postulated, for example, in the prevention of the paralysis associated with iatrogenic injury of medial antebrachial cutaneous nerve during ulnar nerve surgery.

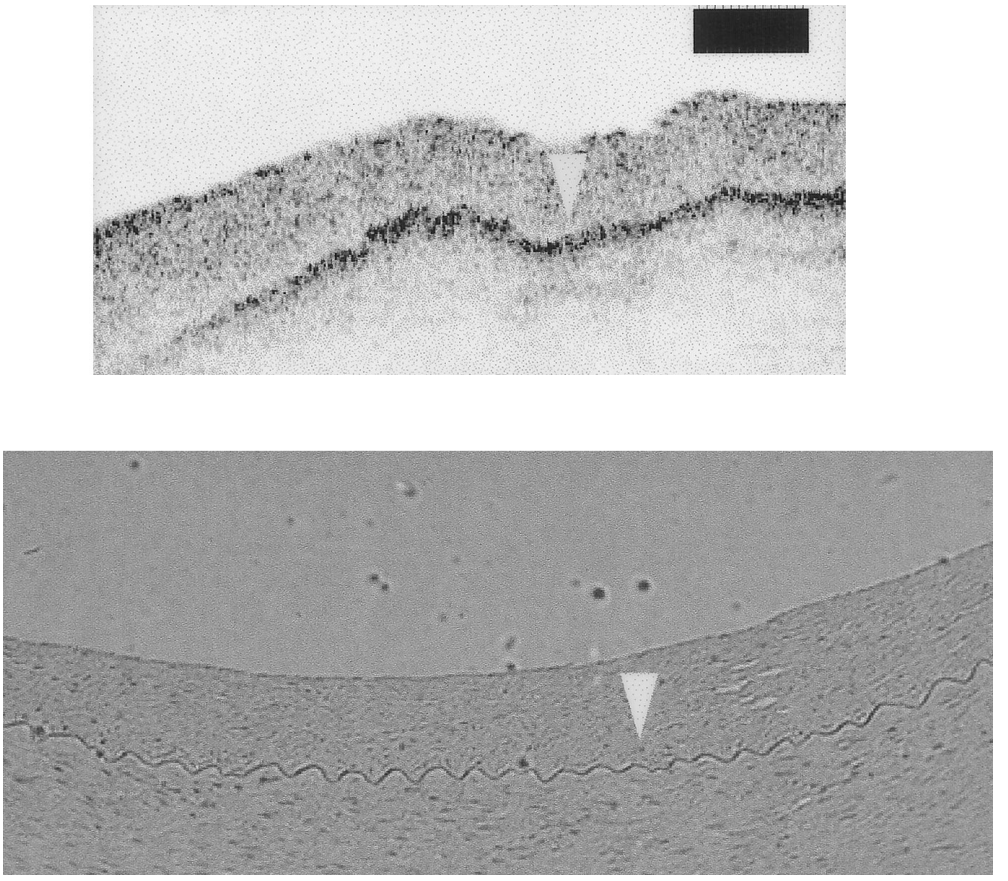

FIG. 7. Renal artery. An image of the renal artery is noted. The basement membrane of the artery is clearly identified (arrowhead). Bar represents 500 μm. Corresponding histology has been included.

Vascular

The success rate for the reanastomoses of severed blood vessels has improved significantly over the past five decades [2, 15]. However, the repair of the small vessels continues to have a high complication rate due to such technical difficulties as intimal flaps and adventitial invesion [16]. The ability to perform transmural imaging of small vessel integrity could substantially reduce the morbidity associated with repair. The ability of OCT to perform high-resolution transmural imaging is shown in Figs. 5 and 6. In Fig. 5, a cross-sectional image of a branch of the superior mesenteric artery is shown with a sharp demarcation of the intima, media, and adventitia. Of note, imaging is possible through the vessel to the coverslip below. Figure 6 shows a branch of the inferior mesenteric artery partially embedded in adipose. The external elastic membrane is noted in this image. The images of the right carotid artery in Fig. 7 and renal artery in Fig. 8 illustrate the ability of OCT to identify fine microstructure, in particular the internal elastic membrane (arrowhead).

Reproductive

Microsurgical interventions designed to restore fertility in either the male or female reproductive tract would likely benefit from high-resolution subsurface imaging, since anatomy and pathology are frequently oriented on a microscopic scale. In Fig. 9, a cross-sectional image of the proximal (a) and distal oviduct (b) is demonstrated, with corresponding histology (c). The fine surface structure, as well as underlying loose connective tissue, is well differentiated.

DISCUSSION

Technologies currently used to guide and assess fine surgical procedures include loupe magnification, microscopy, and ultrasound [17, 18]. Galilean and Kepler loupes are devices which magnify the surgical field up to a factor of 8. They are widely utilized, in part due to the ease with which they can be applied during surgical procedures. Their disadvantages are the limited magnification, the need for sufficient light in the operating field, and inability to provide information about structure within tissue. The introduction of microscopy into the operating field led to significant improvements in the available magnification [17, 18]. However, operative microscopes are difficult and time consuming to set up in the surgical field and have a limited area of view. A built-in light source improves visibility within the operative field but shadowing of structural details cannot be completely prevented. Furthermore, micros-

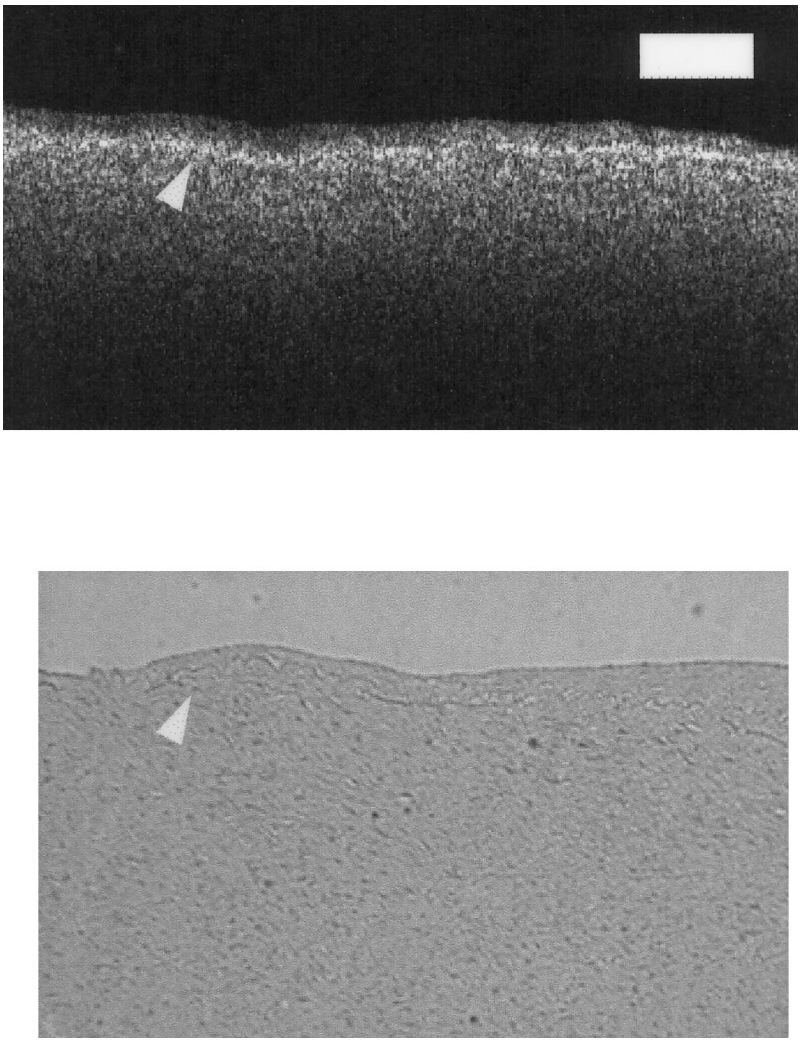

FIG. 8. Carotid artery. An image of the right carotid artery is shown. The basement membrane (arrowhead) is noted in the image. Bar represents 500 μm. Corresponding histology has been included.

copy also does not provide information about structure below the tissue surface. High-frequency ultrasound has recently been suggested for the guidance of surgical procedures [19, 20]. Its advantages are its high resolution, ability to provide information about the internal microstructure of tissue, and the independence from external lighting. The disadvantages are the relatively large transducer present in the operating field and the requirement of a transducing medium. We have previously demonstrated the superior quantitative and qualitative resolution of OCT when compared with a 30-MHz transducer (16 $\pm$ 1 μm for OCT and 110 $\pm$ 7 μm for ultrasound) [21].

In this work, we suggest a feasibility of optical coherence tomography for surgical guidance and diagnostics by demonstrating that high-resolution imaging can be obtained in tissue with important surgical implications. Tissue was selected from the nervous, reproductive, and vascular systems since microsurgical interventions have become increasingly important in these organ systems. Issues which remain the subject of future investigations include optimizing the system for use in the operative field and exploring the ultimate utility of high-resolution imaging to the surgeon and patient.

Future modifications will focus on reducing the acquisition time, increasing the axial resolution, and integrating the system with surgical instrumentation. The acquisition times used in this study (10 sec, Figs. 4a and 4d; 15 sec, Figs. 7 and 8; 30 sec, Figs. 3, 5, 6, and 9; 45 sec, Figs. 2 and 4c) are not adequate for *in vivo* imaging. However, recent advances have led to acquisition rates of 250 msec/image (without a reduction in resolution) and substantially faster rates are likely to result with future engineering modifications [22]. The axial resolution of the OCT system used in this study was 16 μm, higher than any current clinical imaging technology. This resolution has recently been increased through the use of broader bandwidth sources to 4 μm, which allows a resolution at the subcellular level [6]. Finally, since OCT is based on technology used in optical communications, it can be readily integrated with surgical instruments, microscopes, and endoscopes. Similar technology has been used in an OCT catheter designed for vascular imaging [23].

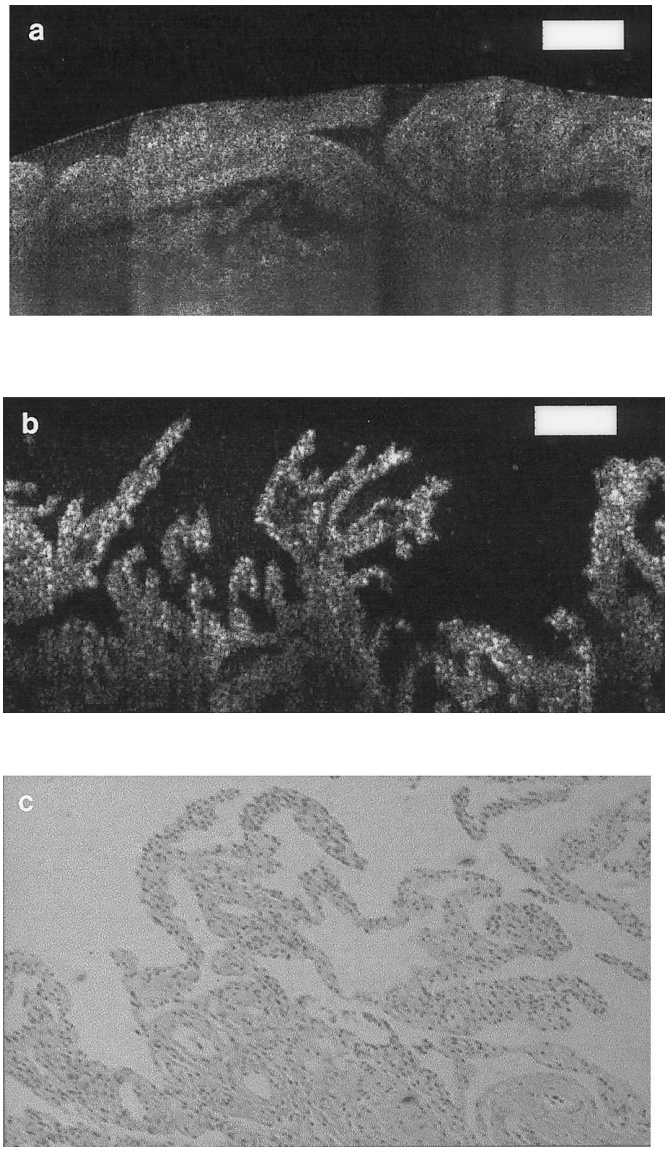

FIG. 9. Oviduct. OCT images of the distal (a) and proximal (b) oviduct, in addition to the corresponding histology of the latter (c), are shown. In addition to the high-resolution imaging of fine surface features, subsurface structure consistent with loose connective tissue regions is seen. Bar represents 500 μm.

The studies in this preliminary work demonstrate the feasibility of OCT for surgical imaging through work on *in vitro* postmortem samples. Future investigations will focus on defining the ultimate utility of OCT to surgical intervention, initially by assessing the ability of OCT to define both microstructure and pathology *in vivo*. Later studies will need to evaluate the ultimate utility of cellular level resolution to the surgeon and patient, since no comparable imaging technology exists in clinical use.

In conclusion, OCT is an attractive new technology for surgical imaging due to its high resolution, compact portable design, low profile, and optical fiber-based construction. In this work, the feasibility of OCT for surgical imaging was suggested by demonstrating that micrometer-scale imaging is possible in surgically relevent tissue. Future studies are merited which examine the impact of micrometer-scale surgical guidance and diagnostics on patient morbidity and mortality.

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