Instruments & Methods

High-resolution imaging of gynecologic neoplasms using optical coherence tomography

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***Background*: A modality capable of imaging the female reproductive tract, at or near the cellular level, could lead to the detection of diseases at earlier stages than currently possible. Optical coherenece tomography achieves high resolutions in the cellular range (4–20 μm) and could accomplish that level of detection.**

***Method*: Optical coherence tomography imaging of gynecologic tissue was studied in vitro on normal and neoplastic human cervical and uterine tissue.**

***Experience*: The structures of the normal ectocervix and endocervix, including epithelium, basal membrane, and glands, were identified clearly. These findings were compared with changes associated with carcinoma in situ and invasive carcinoma. The optical coherence tomography images of the uterus also showed changes between microstructural features of normal tissue and endometrial adenocarcinoma.**

***Conclusion*: Optical coherence tomography of tissue microstructures showed potential for powerful, minimally invasive assessment of the female reproductive tract at a resolution greater than any current clinical imaging method. (Obstet Gynecol 1999;93:135–9. © 1999 by The American College of Obstetricians and Gynecologists.)**

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Diagnostic imaging methods available to gynecologists include magnetic resonance imaging (MRI), ultrasonography, and direct fiber optic endoscopy. These technologies assess a wide range of female reproductive tract disorders for treatment; however, many diseases, such as early neoplastic changes, remain beyond their detection limits. A modality capable of imaging the female reproductive tract at or near the cellular level could detect disease at earlier stages than currently possible. The current clinical technology with the greatest resolution, high-frequency ultrasonography (30 MHz), has a maximal resolution of 110 μm but is insufficient to identify epithelium or other microstructures.[1,2] Optical coherence tomography, an imaging technology developed recently, has achieved resolutions in the cellular and subcellular range (4–20 μm), which could improve the diagnostic range of clinical imaging procedures.[3,4]

Optical coherence tomography measures the intensity of back-reflected infrared light the way ultrasound measures reflected sound waves, using low-coherence laser light to generate two- and three-dimensional images of tissue microstructure on a micrometer scale.[4] Imaging is done at high resolution over the same approximate distance as a conventional biopsy (2–3 mm). In addition to high resolution, several features of optical coherence tomography make it a powerful tool for diagnosing many gynecologic diseases. Imaging can be at or near real time, with disease of the reproductive tract monitored on-screen and stored on high-resolution video tape. It is compact and portable, important for a clinically viable device, and unlike ultrasound, imaging is done directly through air, without direct tissue contact or a transducing medium. Because optical coherence tomography is fiber optic-based, imaging is done through small optical coherence tomography endoscopes (less than 1 mm diameter) or integration with existing endoscopes.

Optical coherence tomography was first used to image transparent structures such as anterior eye and retina.[5,6] Since then, it has advanced to high-resolution imaging of nontransparent tissue, including identification of diseases in the cardiovascular system, gastrointestinal tract, skin, and nervous system.[7–10] Recently, in vivo endoscope-catheter-based imaging has been done at four frames per second of rabbit esophagus, bronchial system, and aorta.[11,12]

In the present article, feasibility of optical coherence tomography for high-resolution imaging of gynecologic tissue is studied on human cervical and uterine tissue in vitro. Normal and diseased microstructures were imaged at a resolution higher than any currently available noninvasive, clinical imaging technology, and the images were correlated with histopathology to confirm tissue identity.

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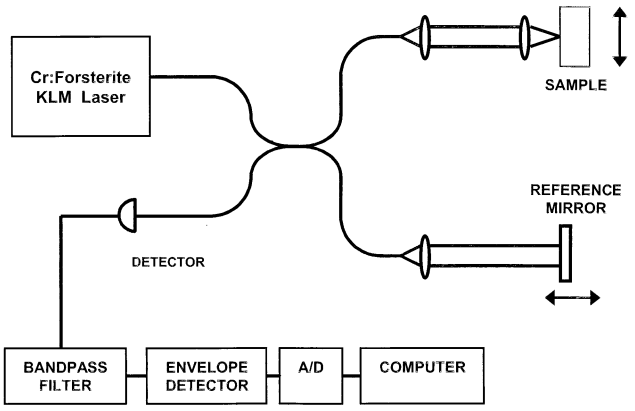

Figure 1. Schematic diagram of optical coherence tomography system.

Method

A schematic diagram of an optical coherence tomography system is shown in Figure 1. Imaging is done by directing low-coherence laser light at the sample and detecting reflections from various internal structures similar to the way ultrasound measures reflected sound waves. Because of the speed of light, direct electronic measurement of the echo delay time of the reflected light is impossible; therefore, a technique called low-coherence interferometry is used. Within the interferometer, the beam leaving the light source is split into two parts, a reference and a sample beam. The reference beam is reflected off a mirror at a known distance and returns to the detector. The sample beam reflects off different layers of tissue, and the two beams recombine. Light that returns to the detector simultaneously from both arms, having traveled the same path length, interferes when it reaches the detector. Optical coherence tomography measures the intensity of this interference. By changing the distance light travels in the reference arm, using a moving mirror that changes the optical path length, the echo time delay and intensity of back-scattered light from the tissue can be measured. Two- or three-dimensional images are produced by scanning the beam across the sample and recording the optical back-scattering versus depth at different transverse positions.

Axial resolution of optical coherence tomography is defined by the coherence length of the light source, which is inversely proportional to the bandwidth (distribution of wavelength within beam) of the light incident. A short-pulse chromium-forsterite laser with a center wavelength of 1280 nm with a bandwidth of 130 nm was used. The bandwidth of the light source yields a 6-μm axial resolution.[13] The 1280-nm wavelength in the near-infrared is close to a minimum in tissue absorption, thus allowing deep penetration and imaging. The transverse resolution is limited by the optical characteristics of the delivery system on the specimen and, more specifically, by the numerical aperture of the lens and the optical wavelength of the incident light, as in conventional microscopy. The combination of optics and source used for the experiments reported here results in a 10-μm transverse resolution.

Experience

Normal and neoplastic uterine and cervical tissue was obtained from surgically excised specimens and imaged fresh. Sixty-five sites from seven different patients were imaged for this study (cervix: 21 normal, two neoplastic

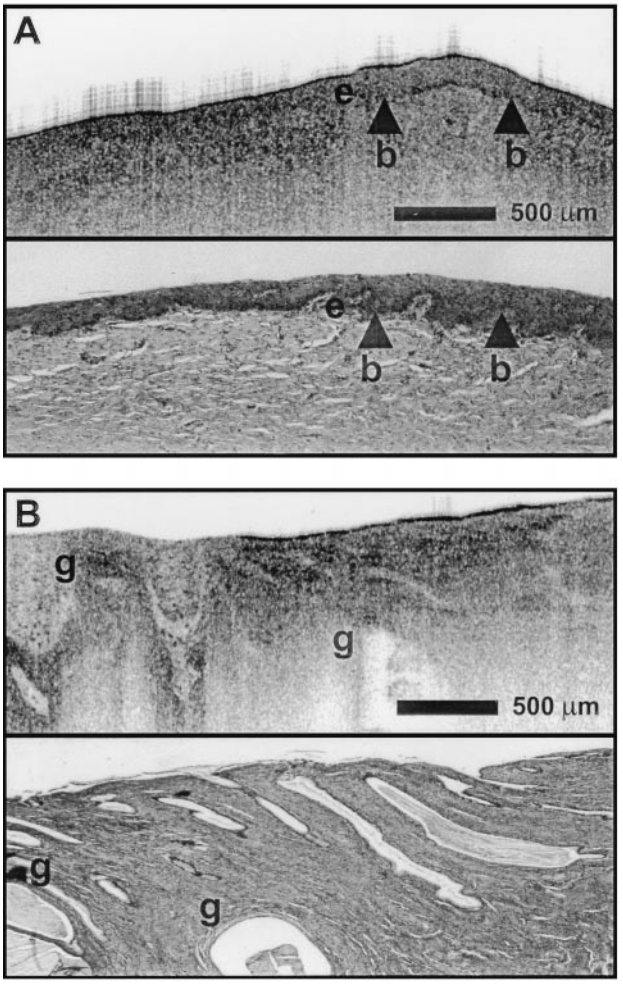

Figure 2. Human cervix imaged in vitro (image size: 1.5 mm × 2 mm; resolution: 6 μm × 10 μm). A) Microstructure associated with the ectocervix: epithelial layer (e) and basal membrane (b). B) Microstructure associated with the ectocervix: deep endocervical glands (g), some of which developed into fluid-filled cysts. The epithelial layer has been partially denuded in the histologic photograph.

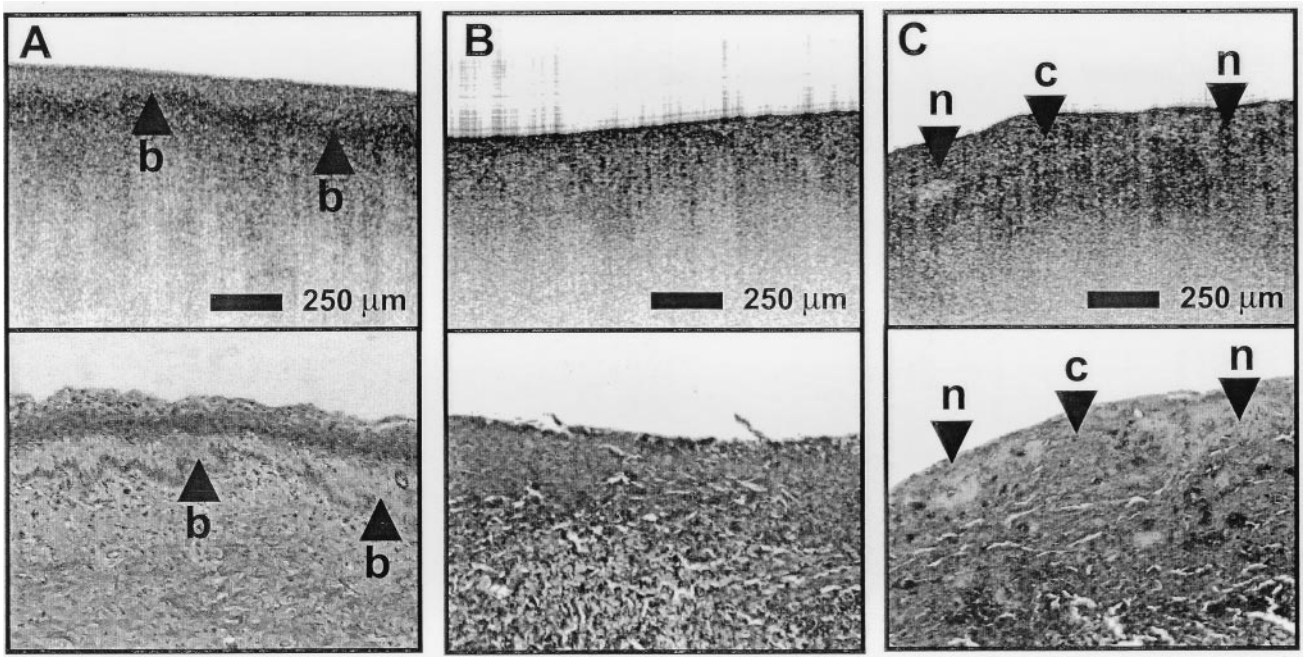

Figure 3. Cervical diseases imaged in vitro (image size: 3 mm × 2 mm; resolution: 6 μm × 10 μm). A) Carcinoma in situ, characterized by a thick, irregular epithelial layer and thickening of the basement membrane (b). B, C) Invasive carcinoma. The tissue surface is now heterogeneous, and the basement membrane is no longer defined. Distinct back-scattering patterns can be seen in cellular (c) and noncellular (n) regions.

in situ, and nine carcinoma; uterus: 11 normal, 22 adenocarcinoma). Tissue specimens were placed in a petri dish and irrigated with isotonic saline to prevent dehydration during imaging. Imaging was performed using a bench-top system similar to the one shown in Figure 1. The acquisition of each image required between 10 and 30 seconds, depending on the size (number of pixel elements) of the image. Because the optical coherence tomography beam is invisible, tissue was registered with a visible-light guiding beam. The orientation of the imaging scan was marked on the specimen using India ink microapplication. There are small variations between the cross-sectional plane of imaged tissue and the plane sectioned histologically. Slight mismatching between histology and imaging plains, and changes in physical dimensions of the tissue, associated with fixation and sectioning, account for the minor differences observed. The samples then had routine histologic processing, were immersed in 10% buffered formalin for 48 hours, and were processed for standard paraffin embedding. Five-micrometer-thick sections were cut at the marked imaging sites and were stained with hematoxylin and eosin. The stained histologic sections verified tissue identity and identification of sources of tissue contrast in the optical coherence tomography images.

Human cervix was imaged in vitro using optical coherence tomography at a resolution of 6 μm. In Figure 2, microstructure associated with the ectocervix and the endocervix was shown. The epithelial layer of the ectocervix and the basal membrane were identified clearly. Deep endocervical glands, some of which developed into fluid-filled cysts, also were visible and are a common finding in postmenopausal women. In Figure 3, cervical samples with carcinoma in situ and poorly differentiated carcinoma were examined. In Figure 3A, the carcinoma in situ is characterized by a thick, irregular epithelial layer and thickening of the basement membrane. With invasive carcinoma (Figures 3B and 3C), the tissue surface is heterogeneous and the basement membrane is no longer defined. Images were correlated with histopathology.

Figure 4 demonstrates in vitro optical coherence tomography imaging of postmenopausal endometrium. The images show sparse uterine glands, consistent with postmenopausal uterine atrophy, which has been confirmed by histopathology. In Figure 4B, larger fluid-filled cysts can be seen. In vitro optical coherence tomography images of neoplastic changes in the uterine endometrial adenocarcinoma are shown in Figure 5. In Figures 5A and 5B, defined epithelial layers and glands are no longer present. The interlacing of cellular and noncellular tissue results in a layered appearance, with the presence of a rare hyperplastic gland.

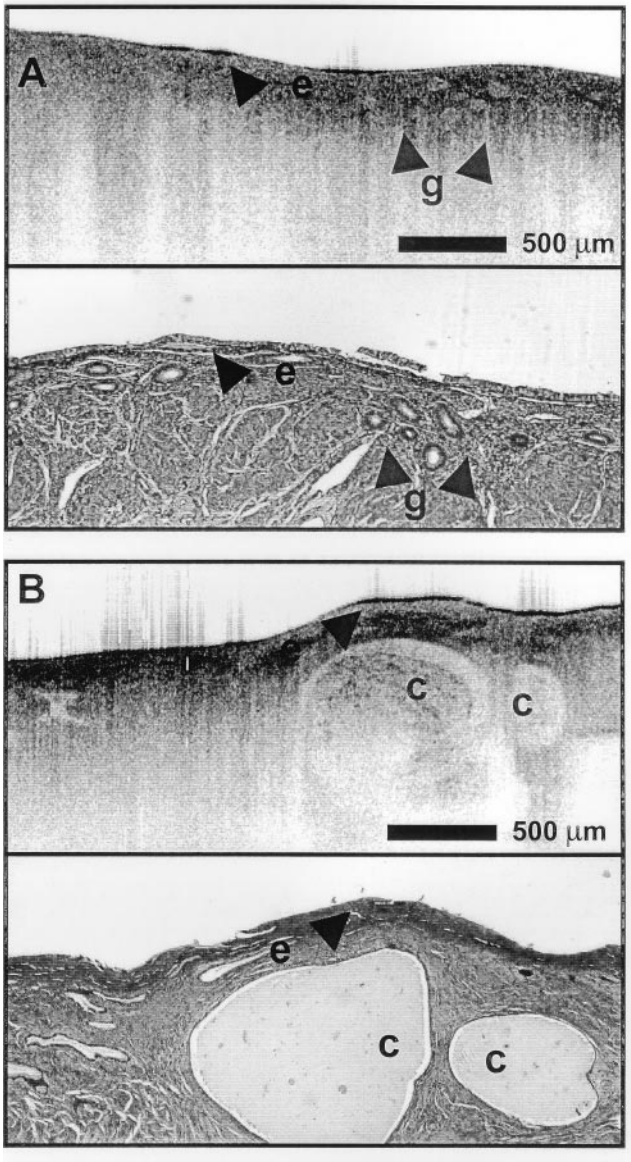

Figure 4. Postmenopausal endometrium imaged in vitro (image size: 3 mm × 2 mm; resolution: 6 μm × 10 μm). A&B) Sparse uterine glands (g), consistent with postmenopausal uterine atrophy; epithelial layer (e); and larger fluid-filled cysts (c) can be identified.

Comment

This study shows the feasibility of optical coherence tomography for imaging clinically relevant microstructures of uterus and cervix. Neoplastic lesions of the uterus and cervix were examined because of their clinical implications. Sharp differentiation of structures included epithelium, glands, supportive tissue, and intramural cysts. The recent demonstration of fiberoptic endoscope-based real-time optical coherence tomography in vivo in an animal model suggests the ultimate feasibility of applying this technique to the female human reproductive tract.[11]

By integrating optical coherence tomography with small imaging endoscopes, the uterine surface could be scanned and diseases imaged in situ at high resolution in real time. This shows potential for screening patients for endometrial carcinoma. Endometrial cancer is one of the most common neoplasms of women in industrialized societies. The majority of patients have postmenopausal bleeding and are diagnosed at early stages. Unfortunately, 90% of dilation and curettage procedures performed for postmenopausal bleeding show no

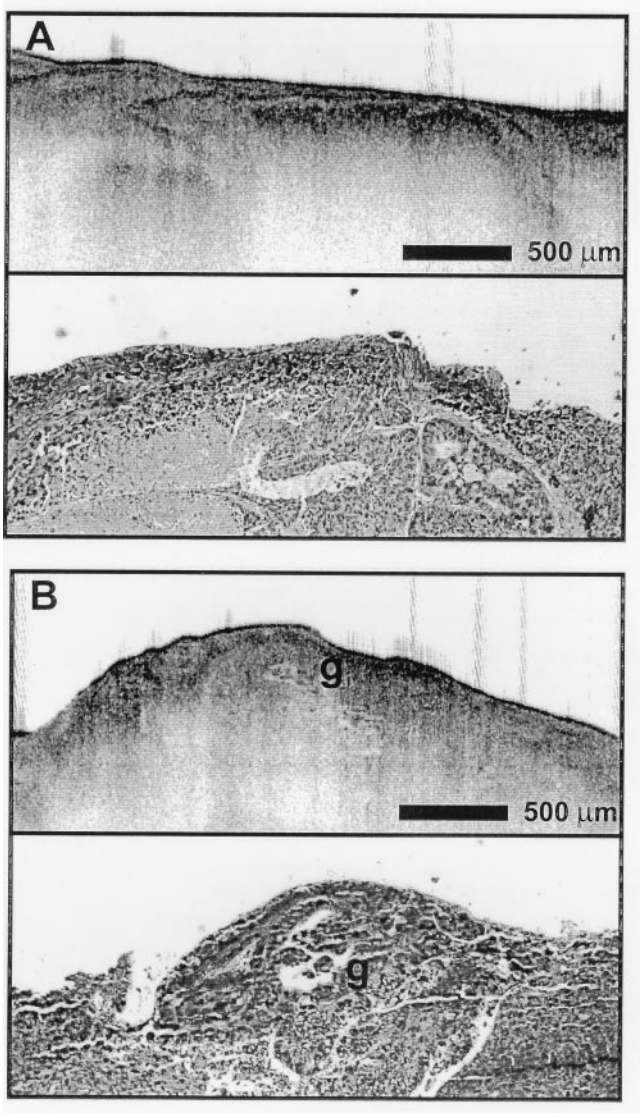

Figure 5. Endometrial adenocarcinoma imaged in vitro (image size: 3 mm × 2 mm; resolution: 6 μm × 10 μm). A, B) Defined epithelial layers and glands are no longer present. The interlacing of cellular and noncellular tissue results in a layered appearance, with the presence of a rare hyperplastic gland (g).

abnormality. A minimally invasive technique with high sensitivity and low false-positive rate would be useful in selecting which patients would benefit from invasive investigation. This is particularly relevant to patients on tamoxifen, who show an increased incidence of endometrial abnormalities (6.3 per 1000 women [at 5 years]), an increase of 2–3 times over the general population.[14] A screening approach is needed for these women, especially if therapy will be extended prophylactically to patients at high risk for breast cancer. Current recommendations leave screening to the discretion of individual gynecologists.[15]

Improvements in endoscope design, acquisition rates, and resolution are necessary to transform the current optical coherence tomography system into a viable clinical device. Optical coherence tomography endoscopes will require only an optical fiber and passive light-directing elements, making them relatively inexpensive. The prototype catheter-endoscope used in previous in vivo animal studies is 1 mm or a 2.9 French in diameter, but ultimately could be engineered to be considerably smaller. Optical coherence tomography endoscopes could be designed and used in conjunction with existing endoscopes or through rigid laproscopes.

The acquisition rate for images in this study ranged from 10–30 seconds per image. This is obviously too slow for routine clinical use. Recent optical coherence tomography systems have been developed that can generate images at 4–8 frames per second.[12] With future modifications, acquisition rates at or near video rate are very likely. The 6-μm resolution of images in the present study allows imaging of tissue microstructures, but does not allow subcellular imaging. The ability to identify individual cells and assess subcellular structures such as the nuclei would be useful in assessing a wide range of neoplastic disorders. Recently, in vivo cellular-level optical coherence tomography imaging in developmental biology specimens was shown by using other solid-state laser light sources.[4] Although additional advances are required to show cellular-level imaging in humans, further improvements in technology and performance might be expected in the future.

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