Imaging the internal structure of the rat cochlea using optical coherence tomography at 0.827 μm and 1.3 μm

BRIAN J. F. WONG, MD, YONGHUA ZHAO, PHD,* MARK YAMAGUCHI, BS,† NADER NASSIF,‡ ZHONGPING CHEN, PHD, and **JOHANNES F. DE BOER, PHD,** Irvine and Orange, California, and Boston, Massachusetts

OBJECTIVE: **The purpose of this study was to use high-speed optical coherence tomography (OCT) to obtain cross-sectional images of the rat cochlea.**
STUDY DESIGN AND METHODS: **The cochleae of Sprague- Dawley rats were imaged within 2 to 4 hours after death. Specimens were prepared by removing the bulla to expose the mesotympanum; some images were obtained in intact temporal bones removed from the cranium. The central element of an OCT imaging device is a Michelson interferometer combined with a low-coherence light source. This study used 2 OCT systems with different light sources: 1) a semiconductor optical amplifier operating and 2) a superluminescent diode with peak emissions wavelengths of 1.3 μm and 827 nm, respectively. Images (1.87 × 2.00 mm or 1.87 × 1.00 mm, 10 × 10 μm pixel resolution) were acquired at a frame rate of 1 Hz. Cross-sectional images at 100-μm increments were obtained from a medial-to-lateral direction.**
RESULTS: **The scala vestibuli, scala media, scala tympani, modiolus, spiral ligament, and several turns of the cochlea were identified.**
CONCLUSION: **These images reflect the ability of OCT to provide images of the internal cochlea structure with micron scale resolution and at near-real time frame rates.** (Otolaryngol Head Neck Surg 2004;130:334-38.)

Optical coherence tomography (OCT) is an emerging imaging modality that uses a Michelson interferometer combined with a low-coherence light source to image the location and variation in tissue optical scattering strength.[1,2] OCT is analogous to ultrasound except light is used instead of acoustic waves. The technique is noncontact with high sensitivity and spatial resolution on the order of 10 μm. Previously, we reported the use of OCT to image the cochlea in ex vivo rat temporal bones using a cumbersome air-space device with slow frame rates (approximately 5 to 15 minutes per frame).[3] In this study, we use 2 novel fast fiberoptic-based OCT imaging system incorporating an 827-nm and a 1.310-μm low coherence source[4] and a rapid spectral optical delay (RSOD) line[5] to obtain serial tomographic sections through the rat cochlea in both in situ and ex vivo temporal bones.

MATERIALS AND METHODS

OCT Instrumentation

Two different fast fiberoptic-based OCT systems were used to provide cross-sectional optical images of the cochlea. The fundamental principles of OCT are described in Huang et al[1] and Tearney et al.[2] The 1.310-μm system allows greater penetration and less signal reduction due multiple scattering than at the shorter wavelengths; the power

From the Beckman Laser Institute and Medical Clinic, University of California Irvine in Irvine (Drs Wong, Yonghua, and Chen and Mssrs Yamaguchi and Nasif), Department of Otolaryngology–Head and Neck Surgery, University of California Irvine in Orange (Dr Wong), Department of Biomedical Engineering, Samueli School of Engineering, University of California Irvine in Irvine (Drs Wong and Chen), and Wellman Laboratories of Photomedicine, Massachusetts General Hospital, Harvard Medical School (Dr de Boer).

Presented at the Western Section Meeting of the Triological Society, January 7, 2001, La Costa Resort and Spa, Carlsbad, CA.

This work was supported in part by the National Organization for Hearing Research, National Institutes of Health (DC 00170-01, 1 r24EY12877-01, GM 58785), Office of Naval Research, Air Force Office of Scientific Research, the Department of Energy, and the Whitaker Foundation (WF-26083). The authors wish to acknowledge the support of the Beckman Foundation and the UC Irvine Health Sciences Partners.

*Dr Zhao is currently with Carl Zeiss Meditec Inc, Dublin, CA.

†Dr Yamaguchi joins the Department of Anesthesiology at the University of Illinois, Chicago in July 2004.

‡Mr Nassif is currently at Harvard Medical School, Boston, MA.

Reprint requests: Brian J. F. Wong, MD, Beckman Laser Institute and Medical Clinic, University of California Irvine, 1002 Health Sciences Rd E, Irvine, CA 92612; e-mail, bjfwong@bli.uci.edu.

0194-5998/$30.00

doi:10.1016/j.otohns.2003.11.007

of this source is 10 times greater than the 856-nm system used in our earlier studies,[3] albeit with a slight reduction in image contrast. This wavelength is optimal for imaging the tissue structure in turbid media such as skin and bone. Image size is 1.9 mm laterally (20-μm resolution) and 2 mm in depth (10-μm resolution). The 827-nm system was originally developed and optimized for use in retinal applications and is a modification of the system described by Zhao et al,[6] differing only in the central wavelength of the low coherence source. In general, the shorter wavelength source results in a reduction in optical penetration depth in exchange for a gain in image contrast; images for the 827-nm system were 1.97 mm laterally × 1 mm in depth. Approximately 1.5 seconds is required to record 1 image frame for both devices. The construction and design of these 2 OCT images systems and the RSOD are described in detail elsewhere.[4,6]

Specimen Preparation

Five Sprague-Dawley rats (~250 g) were obtained immediately after death for use in other protocols approved by the Institutional Animal Care and Use Committee at the University of California Irvine. For ex vivo imaging studies, soft tissue was removed from the cranium and the temporal bones were extracted and stored in saline until use. For in situ imaging studies, a postauricular incision was used to expose the mastoid bulla. Surrounding soft tissue and bone over the middle ear space were removed to provide access to the promontory (and cochlea within).

Imaging Studies

Specimens were secured to a 3-axis micropositioning stage, which allowed positioning of the cochlea relative to the fixed optical path of the imaging systems. Cross-sectional images were obtained at 100-μmm intervals through the cochlea from a medial-to-lateral direction.

RESULTS

Figures 1 and 2 are optical sections through the cochleae of 2 different rats using the 1.310-μmm OCT system. The penetration depth of this wavelength in turbid media (such as biological tissues) varies from approximately 0.5 to 2 mm. The bone of the promontory is an optical dense tissue that absorbs and scatters a significant fraction of the incident source signal, resulting in the reduction in signal intensity with depth. The images in Figure 1 were obtained in an extracted temporal bone. The osseous anatomy of the promontory (indicated by open arrow) is well demarcated with the identification of several turns including the helicotrema (Fig 1, a-c). There are several linear structures in the apical turns with high signal intensity (see filled arrow), which suggest the basilar and Reissner membranes (Fig 1, d-g). The spiral ligament (indicated by arrowhead) is well visualized with a distinct crescent-shaped structure adjacent to promontory bone (Fig 1, h-l). High signal intensity in the lower part of images m through p are due to modiolar bone (asterisk). The arrows and symbols used to annotate structures in Figure 1 are also used for Figures 2 and 3. The images compiled in Figure 2 were recorded in situ. Similar structural features are observed in images a through l, which were obtained through a more central midmodiolar section. In these sequences, no membranes were clearly identified. However, the pointed bony structure observed in b through h likely represents the osseous attachment of the basilar membrane (in rats) and marks the division of the scala tympani from the scala vestibuli. Again, the crescent shaped spiral ligament is identified in images I through l. Light from 1.310-μmm sources allows greater discrimination of internal cochlear structure due to deeper penetration than those obtained using the 827-nm system. Images obtained using the 827-nm system are shown in Figure 3. In general, penetration depth is reduced, in exchange for improved image contrast. The thin mucosal layer over the promontory (indicated by stealth arrow) is identified and a distinct line separates this tissue from the underlying bone (a-d). The demarcation between the osseous and membranous labyrinth is clearly identified, and again the spiral ligament is seen in e through p in 2 turns. Despite clear identification of this structure, the overlying stria vascularis could not be distinguished.

DISCUSSION

The first studies to image the rat cochlea with OCT used a slow air-space optical system with a

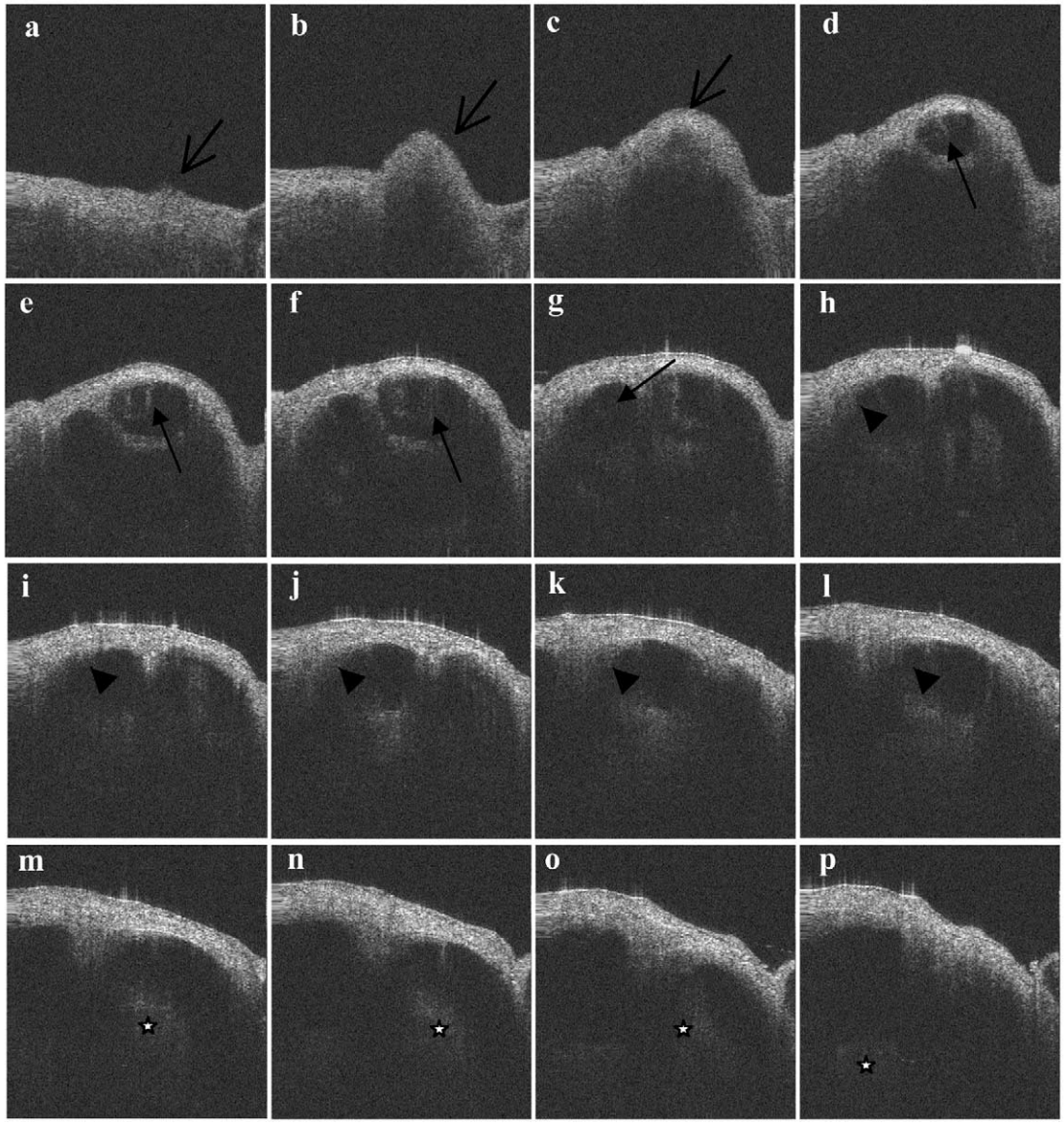

Fig 1.•••

low-coherence source centered at 856 nm.[7] Image acquisition time (per scan) was in excess of 5 minutes and penetration depth was limited due to source wavelength and power. The 2 instruments used in this study replaced this bulky system with a fiberoptic-based device incorporating a rapid spectral optical delay line and higher power sources. The 1310-nm source allowed deeper penetration of turbid media and rapid data acquisition (~1 second per frame). Using both systems, the anatomic borders of the promontory, distinct turns of the cochlea, modiolus, and spiral ligament were clearly identified. The basilar and Reissner membranes were less clearly discriminated. The images are more readily interpreted when presented as a montage of spatially separated sequential images in a manner analogous to computed tomography or magnetic resonance imaging studies as illustrated (Figs 1-3). Of note, the rat cochlea differs from its human counterpart in several respects, most notably in terms of the more scalloped geometry of its turns.[8]

Imaging the rat cochlea with the 827-nm source resulted in reduced depth of penetration as ex-

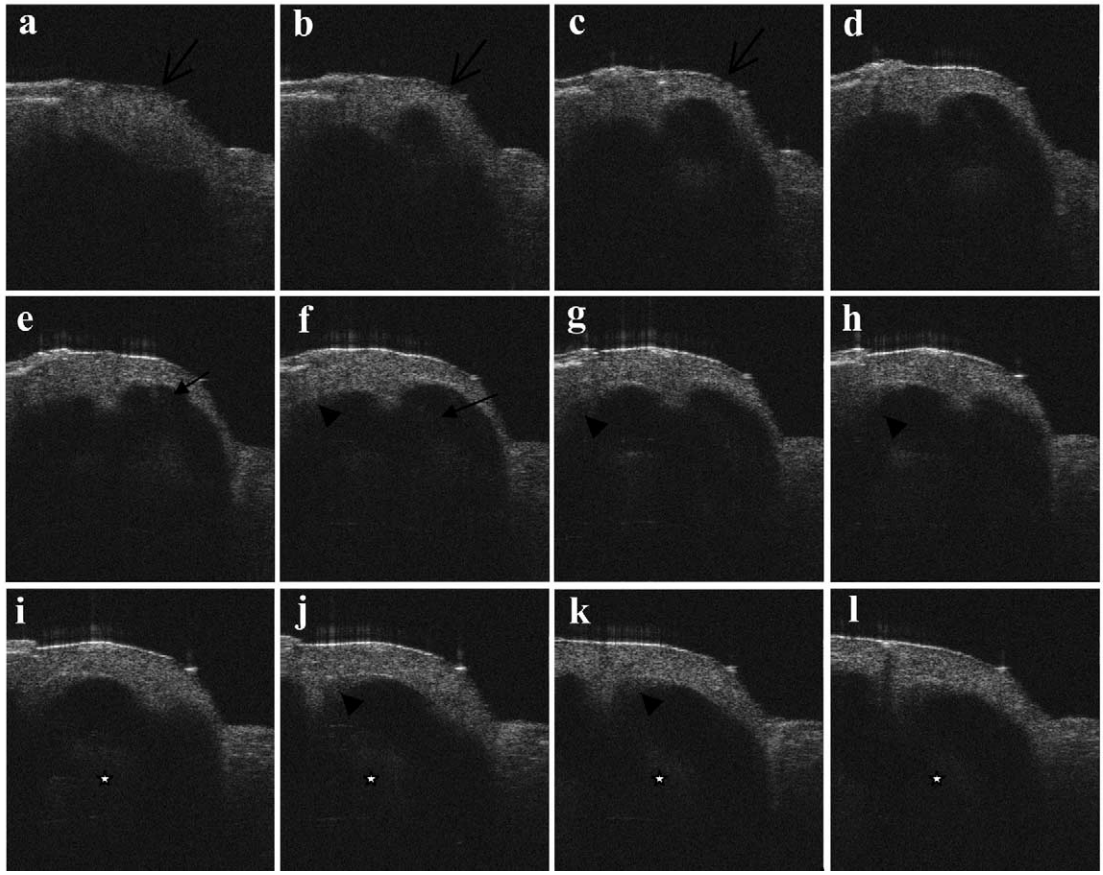

Fig 2. • • •

pected with the identification of fewer internal structures. In fact, apart from the promontory mucosa and spiral ligament, no other membranous anatomy was delineated. This differs from our previous results in imaging the rat cochlea using a slow 856-nm system, where linear structures consistent with the orientation, size, and location of the basilar and Reissner membranes were clearly identified. This can be explained in part by the fundamental difference in optical design between the 2 systems. Our earlier system (air-space) required mechanical translation of the specimen to determine the optical properties of the tissue for each discrete pixel, resulting in integration of the backscattered light signal over long time intervals. In contrast, the present system used the RSOD to achieve the high scan rate (replacing mechanical translation of the specimen). The data acquisition time is shorter by nearly 2 orders of magnitude, resulting in short integration times and reduced signal intensity.

This study demonstrates the feasibility of imaging the cochlea using fast fiberoptic-based OCT imaging systems. At the present, there are no readily available imaging modalities that can provide tomographic information on cochlea structure without biopsy and fixation. While 7T magnetic resonance imaging systems are being developed for research purposes, the cost of these systems will likely preclude widespread use in auditory research laboratories. The capital expenditure for the construction of the present OCT system was approximately $100,000, but commercial OCT systems are currently being manufactured (Light-Light Imaging, Westford, MA; and Zeiss-Humphrey, Dublin, CA), albeit primarily for ophthalmic applications. As a research tool, OCT may provide a means of evaluating structural change in the membranous labyrinth in longitudinal studies within the *same* animal reducing the need for cohort studies. Use of OCT to image the cochlea in humans still remains a technical challenge as

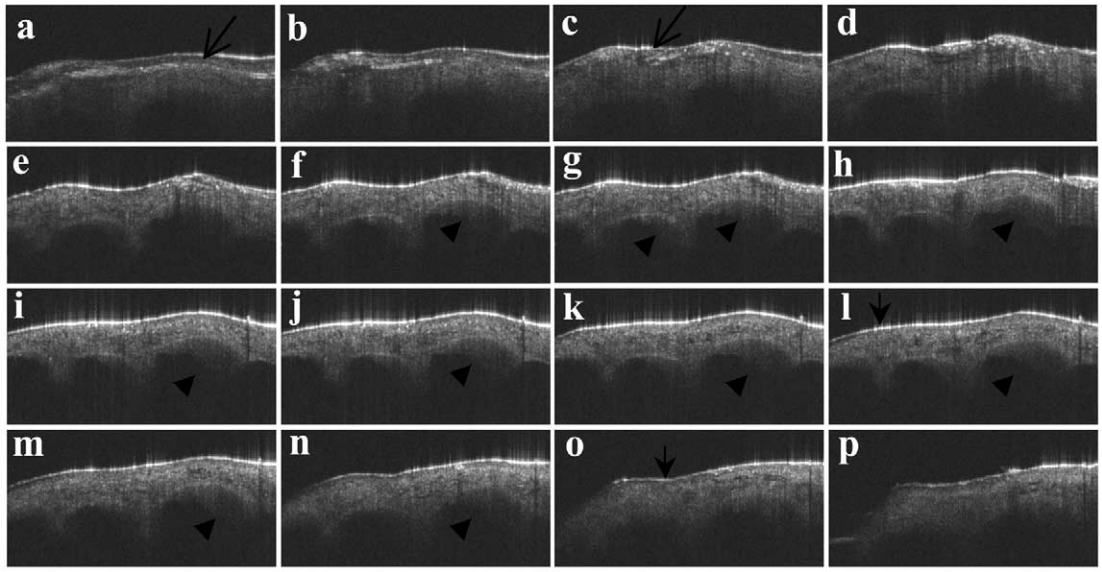

Fig 3. • • •

the thick bone of the promontory results in near total loss of the backscattered light signal.

CONCLUSION

This study demonstrates the feasibility of using fast fiberoptic-based OCT imaging systems for determining cochlea microanatomy with resolution that is limited by the coherence length of the source and optical design of the handpiece (diffraction limited). As this imaging modality is noncontact and uses safe near infrared and infrared light sources, OCT may in the future provide scientists and clinicians a means to image inner ear disorders as higher power sources and faster scanning techniques are developed. At present, an OCT system designed to provide high-resolution images at near real-time frame rates is under construction in our laboratory.

The authors would like to acknowledge the longstanding guidance Thomas E. Milner, PhD, and the technical assistance of Christopher E. Saxer, PhD.

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