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Authors
Wong, Brian J
de Boer, Johannes F
Park, Boris H
et al.

Publication Date
1999-06-22

DOI
10.1117/12.350972

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Peer reviewed
Optical Coherence Tomography of the Rat Cochlea: Preliminary Investigations

Brian J.F. Wong1,2, Johannes F. de Boer1, B. Hyle Park1, Zhongping Chen1, and J. Stuart Nelson1

1Beckman Laser Institute and Medical Clinic, University of California Irvine, Irvine, CA 92612
2Department of Otolaryngology-Head and Neck Surgery, University of California Irvine, Orange, CA 92668

ABSTRACT

Optical coherence tomography (OCT) was used to image the internal structure of a rat cochlea (ex vivo). Immediately following sacrifice, the temporal bone of a Sprague-Dawley rat was harvested. Axial OCT cross-sectional images (over regions of interest, 1 x 1 mm to 2 x 8 mm) were obtained with a spatial resolution of 10-15 μm. The osseous borders of the lateral membranous labyrinth overlying the cochlea and the scala vestibuli, media, and tympani which were well demarcated by the modiolus, Reissner's and the basilar membranes were clearly identified. OCT can be used to image internal structures in the cochlea without violating the osseous labyrinth, and may potentially be used to diagnose inner ear pathology in vivo in both animal and human subjects.

Keywords: cochlea, ear, optical coherence tomography, coherent imaging

1. INTRODUCTION

Optical coherence tomography (OCT) is an evolving imaging modality based on low-coherence interferometry. The first clinical applications were in ophthalmology to image the retina and cornea where OCT has become a standard diagnostic technique. In the past nine years, conventional OCT systems and OCT based endoscopes have imaged structures in the upper airway, vasculature, skin, dentition, gingiva, and nervous system producing cross-sectional images of imbedded anatomic structures with lateral spatial resolution on the order of 10-20 μm. Whereas in magnetic resonance imaging (MRI), computed tomography (CT), and ultrasound reflect differences in protein density, electron density, and elastic modulus, respectively, the OCT signals reflect differences in tissue optical properties. In this study, we used OCT to obtain cross-sectional anatomic images of rat cochlea (inner ear) in an intact temporal bone.

The morphologic features accompanying inner ear disease are difficult to study because biopsy or excision result in the complete loss of hearing. As a consequence, knowledge of human ear pathology is limited to specimens obtained from temporal bone banks which are of value only when the clinical history of the deceased is known in detail. There are limited number of such specimens (approximately 12,000 in the United States) and most specimens have some degree of artifact caused by the delay in temporal bone fixation and processing following death. Hearing research in animals has similar limitations in that morphologic alterations in cochlear structure can only be determined following sacrifice. Hence, to this point longitudinal studies of inner ear pathology in a single animal have not been possible.

While clinicians are able to assess and diagnose cochlear diseases by integrating clinical history with audiologic and electrophysiologic examination, cochlear pathology can not be determined in vivo. Conventional imaging modalities such as magnetic resonance imaging (MRI) and computed tomography (CT) are severely limited by resolution (1 mm), cost, concern over ionizing radiation (CT and microangiography), acoustic trauma (high frequency ultrasound), or inability to distinguish osseous and soft tissues simultaneously (MRI). In this study, our objective was to evaluate the feasibility of OCT for imaging cochlear structure using an ex vivo rat temporal bone.

2. MATERIALS AND METHODS

An intact temporal bone was harvested from a freshly sacrificed adult Sprague-Dawley rat in accordance with the regulations and guidelines of the Institutional Animal Care and Use Committee at the University of California, Irvine. Using microdissection techniques, wide exposure to the promontory and middle ear space was obtained by removing the mastoid bulla. The cochlea lies directly beneath the osseous promontory which is approximately 200 μm in thickness. The specimen was secured to a two-dimensional translational stage and the OCT images were acquired.
OCT is an imaging technique that uses a low-coherence Michelson interferometer to perform optical sections of biological materials (1). The amplitude of the backscattered light is determined by measurement of the interference fringe intensity generated between the reference and the sample arms. High axial spatial resolution is possible because the interference is observed only when the optical path lengths of light in the target and reference arms match to within the source coherence length (2). A schematic of the OCT system used in our experiments is illustrated in Figure 1. The reader should refer to de Boer et al for a detailed description of this apparatus (3, 4).

Continuous near-infrared light emitted by a superluminescent diode (SLD) (0.8 mW output power, central wavelength $\lambda = 856$ nm, spectral FWHM, $\Delta \lambda = 25$ nm) was split into reference and sample arms by a beamsplitter (BS). Light in the reference arm was reflected from a mirror attached to a piezoelectric transducer (PZT) and retroreflected. A carrier frequency (6 kHz) was generated by displacing the PZT driven mirror over 20 $\mu$m with a 50 Hz triangular or 100 Hz sawtooth waveform. The PZT retroreflector assembly was mounted on a translational stage to allow for active focus tracking in the sample (5). For improved signal to noise ratio, a neutral density filter (NDF) positioned in the reference arm reduced intensity noise by a factor of 50 (6). Light in the sample arm passed through both a lens $L$ ($f=50$ mm) and the specimen, and then was retroreflected along the same pathway. The light was recombined in the detection arm of the system and focused ($f = 50$ mm) on a 25 $\mu$m pinhole placed directly in front of a photoreceiver (Model 2001 FC, New Focus, Santa Clara, CA). The resultant optical interference fringe intensity signal was digitized with a 16-bit analog-digital converter and transferred to a computer workstation for processing. A signal was bandpass filtered at the carrier frequency and rectified.

Two dimensional images were formed by lateral movement of the sample at constant velocity $v$ (x-direction) and repeated after each longitudinal displacement (z-direction). Transverse and longitudinal pixel sizes of the images were, respectively, the product of the transverse velocity $v$ and the time duration of a single ramp of the PZT waveform (10 ms for both waveforms), and the longitudinal displacement between transverse scans. Transverse and axial image resolution were 10-15 $\mu$m, and determined by the beam waist at the focal point and the coherence length of the source. Cross sectional OCT images were displayed using software visualization utilities (AVS, Waltham, MA) on a UNIX workstation platform and displayed in greyscale.

3. RESULTS

![Figure 1: Schematic of OCT imaging system: SLD- superluminescent diode, BS- beam splitter, PZT- piezoelectric transducer and mirror, NDF- neutral density filter, and L-lens.](image1.png)

A lateral panoramic OCT cross sectional image of the temporal bone (Figure 2, 2 x 8 mm, 10 $\mu$m/pixel) illustrates three turns of the cochlea, promontory (lateral bone covering the cochlea) and the modiolus (medial osseous core). Image intensity is proportional to the power of the backscattered light in a given region of interest defined by the coherence length.
length of the source (10-15 μm). Due to the high scattering encountered in promontory bone, the signal intensity from deeper structures is small and hence the lower half of the image contains limited morphologic information. In Figure 3 (2 x 2 mm, 10 μm/pixel), only the apical and second turn of the cochlea are imaged. For reference, a schematic of the cochlea (with two turns) is illustrated in Figure 4 using the same labels (promontory P, modiolus M, apical turn at, the second or middle turn mt). The large arrow heads indicate the position of Reissner's membrane while the small arrow heads indicate the location of the basilar membrane (marked for both turns); these structures demarcate the three principal compartments of the inner ear [scala tympani (st), scala media (sm), and scala vestibuli (sv)]. The basilar membrane thickness likely represents structures of the organ of Corti. A higher resolution image of this region of interest is illustrated in Figure 5 (1 x 1 mm, 5 μm/pixel). The internal structures of the apical turn are labeled as in Figure 3.

4. DISCUSSION

Imaging cochlear structure is a novel application of OCT. In Figures 2, 3 and 5, microanatomic features within the cochlear are clearly identified. While these images do not delineate fine structures such as the cochlear hair cells, organ of Corti, or stria vascularis it is clear that the three principal compartments of the inner ear are identified along with Reissner’s membrane and the basilar membrane. While the thin bone overlying the cochlea in rodents permits the imaging of key cochlear structures, the thick bone (>1 mm) covering the human cochlea is highly scattering and the retroreflected light intensity may be extremely small and presents a significant technical limitation. This is evident in Figure 3, where the lower half of the image shows little detail. This is analogous to ultrasound imaging where depth in tissue and the presence of scattering media degrade the propagation of ultrasonic wave.

OCT is a nascent technology, and improvements both image resolution and depth of penetration are forthcoming. Despite the limitations of current OCT technology, useful information about the inner ear structure can still be derived. For example, in Meniere's syndrome (endolymphatic hydrops) Reissner's membrane bulges into the scala tympani in contrast to the linear configuration illustrated in Figures 4 and 5. With our current instrument, we can determine the position of
Reissner's membrane relative to the basilar membrane and scala tympani. In humans, the diagnosis of Meniere's syndrome is based on clinical assessment and only confirmed at the time of autopsy.

Though in both rodents and humans OCT imaging would require access to the middle ear, this does not present a challenge as an OCT probe or endoscopes could be inserted into the middle ear space via a tympanostomy (hole in the eardrum). Tympanostomy, the most common operation in North America, has a low morbidity (<0.5%) rate and is an outpatient clinic procedure performed with local or topical anesthesia.

5. CONCLUSIONS

OCT is an imaging modality that permits in vivo assessment of cochlear anatomy with resolution exceeding conventional CT and MRI by two orders of magnitude. OCT may provide clinicians and scientists with a method to assess the progression of inner ear disease which presently is limited to functional electrophysiological and audiological study. Structural information on cochlear pathology can only be obtained post mortem relying on the procurement of human temporal bones by specialized research centers or the serial sacrifice of large numbers of animal subjects. While in vivo OCT imaging of the human cochlea presents significant technical challenges, imaging rodent cochleae can be accomplished with present technology, and we are presently pursuing research in this area.

6. ACKNOWLEDGMENTS

This work was supported in part by the Office of Naval Research (N00014-94-0874), Whitaker Foundation (WF23281), National Institutes of Health (AR-43419 and RR-01192), American Otological Society, and Department of Energy(95-3800459).

7. REFERENCES


Correspondence: bjfwong@bli.uci.edu; WWW:http://bli.uci.edu; voice:(949) 824-6996, FAX (948) 824-8413