Research Article

Catoptric Objective Based High-Sensitive Fourier-Domain Optical Coherence Tomography

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Abstract

We introduce a novel catoptric objective based high sensitive Fourier Domain Optical Coherence Tomography (FD-OCT) system for enhanced structural and functional imaging of cochlear microstructures. Unlike the conventional refractive type microscopic objective, catoptric objectives are well-known for their chromatic aberrations free operation and high light gathering efficiency for a broad range of wavelengths. In this study, we present the feasibility of a commercially available catoptric type objective to obtain high-sensitivity structural and functional imaging of cochlear microstructures of an excised guinea pig through intact temporal bone.

Introduction

In the past decade there has been a growing interest in using Optical Coherence Tomography (OCT) for basic hearing research and clinical practice [1-5]. The noncontact, noninvasive and high-resolution imaging capabilities of OCT make it as a well suited imaging modality for both middle ear and inner ear studies. Recently, the potential application of OCT for high resolution structural and functional imaging of middle ear has been pursued successfully [3]. In addition, OCT become an emerging technology in the field of fundamental hearing research, where it can be used to image the morphology and function of a rodent's inner ear microstructures to study the cochlear mechanics, and how the travelling wave of the basilar membrane couples its energy to the mechanosensory receptor cells in the Organ of Corti (OC). Freeman, et.al [6] adapted Doppler OCT technique to measure the subnanometer motions of cochlear structures including the basilar membrane, reticular lamina, tectorial membrane, and outer hair cells in the apical turns of a Mongolian gerbil. Using time-domain OCT our group demonstrated the structural and vibration measurements of organ of Corti of the basal turns of a guinea pig in vivo [7]. Using this method we demonstrated that the organ of the Corti lamina in a guinea pig cochlea, in which the vibrations peaked at a higher frequency, had different timing, and were enhanced compared with those at the basilar membrane [8]. In order to improve the acquisition speed and sensitivity the Fourier-Domain based OCT (FD-OCT) is recently adapted into the hearing research field for the functional imaging of inner ear studies [9-11]. However, in all these studies, the specimens were prepared by first opening the auditory bulla to expose the cochlea, and then a hole with a diameter of few hundreds of micrometers was created to allow access of the imaging beam and to provide measurements of high sensitivity. It is a very tedious and time consuming task which often disrupts the delicate inner ear microstructures and directly influences the quality of measurement. Without the hole, the spatial resolution and the sensitivity of OCT measurement are insufficient to provide fine structural features of the cochlear tissues and functional vibration information because of the highly scattering otic capsule bone. In general, the effect of the hole drilled in the otic capsule is to allow the better observation of cochlear organ of the Corti movement. Such measurements are a persistent goal of cochlear mechanics. Methods to avoid making a hole are needed not only because of injury, but the presence of this hole may have mechanical (hydrodynamic) consequences. In order to overcome this limitation, we propose the use of OCT system based on far infra-red optical source with catoptrics type objective to provide high sensitive and high resolution structural and functional imaging of cochlear microstructures through intact temporal bone. Moreover, this is, to the best of our knowledge, the first demonstration of OCT systems based on catoptric objective for both structural and functional imaging application.

Materials and Methods

Optical setup

Figure 1 depicts an experimental setup of catoptrics type microscopic objective based FD OCT system, which includes a fiber-based Michesion type spectral-domain interferometer. A broadband 1310 nm super luminescent diode (94-nm full-width half-max bandwidth, ~10-mW output power, B&WTek Incorporated, Newark, Delaware) was coupled into the interferometer, via an optical
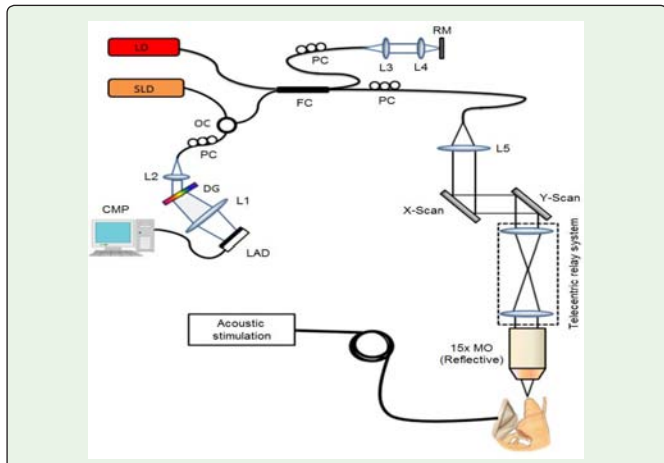
Figure 1: (A) Experimental setup of catoptrics based Fourier-domain OCT system. DG-Diffraction Grating, L1-L5 – Lenses, PC-Polarization Controller, OC-Optical Circulator, FC-Fiber Coupler, LAD- Linear Array Detector, RM-Reference Mirror, MO-Microscopic Objective, CMP-Computer, SLD-Super Luminescent Diode, LD-Laser Diode.

Figure 3: Comparison of measured SDOCT system sensitivity as a function of depth for refractive and catoptric type objective.

coupler. The spectrometer consisted of a 14-bit, 512 pixels InGaAs line scan camera with a maximum acquisition rate of 20 kHz which is adequate for the cochlea apical (low frequency) region where vibrations are below a few kilohertz. This spectrometer setup had a spectral resolution of 0.168 nm, which gave a maximum imaging depth of ~2.5 mm (in air). The measured axial imaging resolution of the system was ~ 8.3 µmin air. The sample arm consisted of a pair of galvanometric driven mirrors and a 15X, NA 0.28 reflective type objective in conjunction with a telecentric relay optics.

The catoptrics objective we used was based on the Schwarzschild configuration available commercially from the Edmund Optics; model NT58-418 (ReflX Objective, Infinite Conjugate, Gold Coating). The measured lateral resolution of the system was ~3 µm with an input beam diameter of 6.5mm. Moreover, this model is supplied with the internal mirrors with gold coating provide a reflectivity of >94% 700-800 nm and >97% 800-2000 nm, which is well matched with our
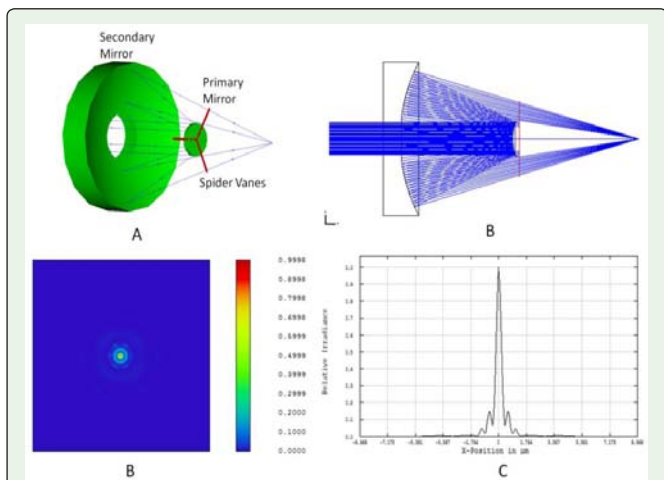
OCT optical source with central wavelength 1310nm. The use of this far infra-red source with OCT can provide enhanced imaging depth because of reduced scattering in biological tissue.

Catoptric objective

Figure 2(A) shows the 3D ZEMAX optical layout of the Schwarzschild configuration based catoptrics objective and Figure 2(B) shows the corresponding 2D optical layout. In contrast to standard refracting microscope objective, catoptric objective are renowned for their unique optical properties such as chromatic aberration free operation and their wavelength independent performance over a broad range of wavelengths. Moreover, additional benefits that can be readily realized with catoptrics objective for OCT applications are their relatively long working distance and high-light gathering efficiency for back reflected light. However, due to the on-axis geometry of the Schwarzschild configuration, the source

Figure 2: (A) 3D ZEMAX optical layout of the Schwarzschild configuration based catoptrics objective (B) corresponding 2D optical layout (C) Simulation of Huygens PSF diagram and (D) corresponding X- and Y-axis cross-sectional Huygens PSF.
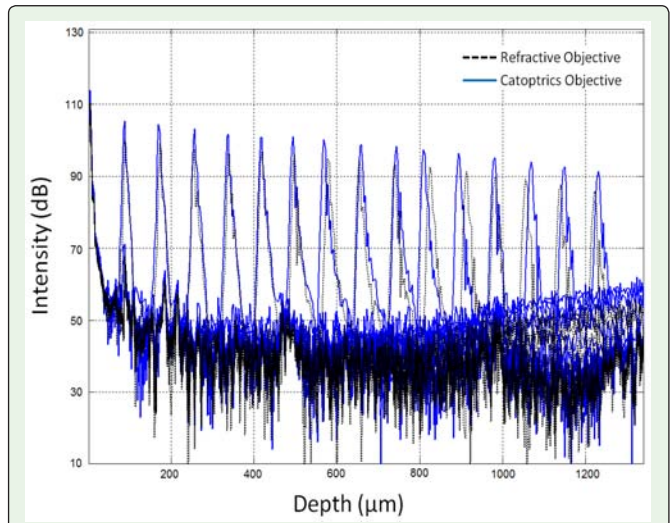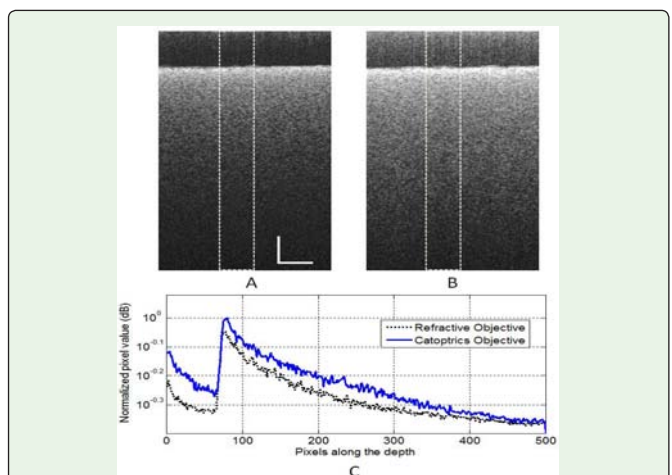

Figure 4: Comparison of OCT images of the reflectance standard same acquired with (A) refractive type objective (B) catoptric type objective. (C) Depth profile of plots of the rectangle region of Figure 4 (A) and (B) (Micrometrebar 200 µm).
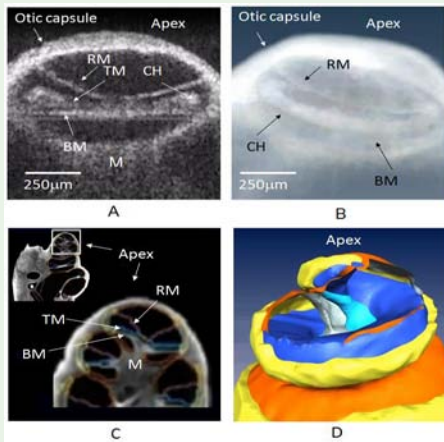
Figure 5: (A) 2D structural cross-section of apical turn of the cochlea through the otic capsule (B) 3D structural view. RM-Reissner's Membrane, BM-Basilar Membrane, TM-Tectorial Membrane, M-Modiolus and CH-Cell Of Hansen (C) corresponding imaging of apex of the cochlea imaged using a technique called OPFOS (Orthogonal-Plane Fluorescence Optical Sectioning) (D) 3D reconstruction by rendering the 2D OPFOS images of the outer wall of the apical turns of cochlea.

light entering the central portion of the primary reflecting surface is reflected back through the input aperture and causes an overall flux or throughput loss. In our case the measured central obscuration loss was ~37%. Figure 2(C) and (D) show the simulated Huygens PSF diagram and corresponding X- and Y-axis cross-sectional Huygens PSF, respectively. The measured PSF of the objective was ~ 3 micron.

Sensitivity comparison

In order to compare the sensitivity performance of catoptrics objective and conventional refractive objective, we did a standard sensitivity measurement of our SD-OCT system with both the objectives. The refractive objective used in this experiment is a 10× NIR coated microscopic objective (Mitutoyo Plan Apo NIR Infinity-Corrected Objective, NA 0.26) with a transmission efficiency of ~ 57% at 1300 nm. To measure the sensitivity, i.e. the smallest possible back reflection which can be detected by our SDOCT system, a fixed reference arm was used in conjunction with an ND filter to control the reference arm illumination. At the sample, a mirror and an ND filter that was stepped by fixed amounts via a linear translation stage with micron step resolution. This method allows for characterization of both falloff as a function of depth and maximum imaging depth.

Figure 3 shows the measured sensitivity falloff as a function of depth for both refractive and catoptric type objectives. The SNR measured at ~ 100 μm from the zero optical path difference point was ~ 98 dB for refractive type objective. However, the catoptric shows an enhanced sensitivity of ~ 107 dB at ~ 100 μm from the zero path length difference. Both these measurements were carried out with a camera exposure time of 0.06ms, which corresponds to a line rate of 11.3 kHz. It can be clearly seen from the Figure 3 that the catoptric objective provides an enhanced sensitivity of ~ 9 dB in compression with the refractive objective. Next, to compare the quality of imaging with catoptric and refractive type objective with our SD-OCT system, we took image sets of a calibrated reflectance standard (WS-1-SL, Labsphere, North Sutton, New Hampshire, USA). Figure 4 (A) and (B) shows the cross sectional images of the reflectance standard with

refractive and catoptric type objective, respectively. Figure 4C shows depth profile plots of the reflectance standard OCT images obtained with both refractive and catoptric type objectives. The depth profile for each type objectives were obtained by averaging 200 A-scans along the lateral direction, which is indicated by the rectangle window in Figure 4(A) and 4(B).

Results and Discussion

To test the imaging capability of our proposed system for our proposed application, we imaged in vitro the apical turns of a freshly prepared excised guinea pig cochlea. A hollow tube was fixed into the ear canal near the tympanic membrane for delivering acoustic stimulation and the other end of the hollow tube is connected to a speaker. The whole preparation was then submerged into a physiological solution media to keep the specimen from drying and measurements were taken over a 15 minutes timeframe after the cochlea was excised.

Figure 5(A) shows the cross sectional image of the structural image from the apical area of the cochlea captured at a frame rate of 15fps. The important anatomical features of the inner ear microstructures at the apical turn are clearly demarcated through the otic capsule, which include the structural features of OC such as Basilar Membrane (BM), Tectorial Membrane (TM), Reissner's Membrane (RM), Modiolus (M) and Cells of Hensen (CH). Figure 5(B) shows the corresponding volumetric reconstruction. Figure 5(C) and (D) shows the corresponding 2D and 3D structural imaging obtained with a histological imaging method called Orthogonal-Plane Fluorescence Optical Sectioning (OPFOS) microscopy [12,14].

In order to test the functional imaging capability, we imaged the vibration amplitude of cochlear microstructures under acoustic stimulation condition. The delivered frequency of the acoustic stimulation was at 500 Hz with a Sound Pressure Level (SPL) of 70 dB. The processing of the vibration detection follows the same algorithm and scanning protocol described in [11]. A MB-mode (M-Scans where acquired over a B-frame at 200 measurement points with a lateral scan range of 900 μm) scanning protocol is employed to acquire data during acoustic stimulation. Each M-scan consists of 1000 A-lines at fixed lateral position on the sample. Figure 6(A) and 6(B) shows the structural image and the corresponding vibrational image, respectively. The vibration map shows the amplitude of motion of various microstructures such as Basilar Membrane (BM), Tectorial Membrane (TM), Reissner's Membrane (RM) and Cells of
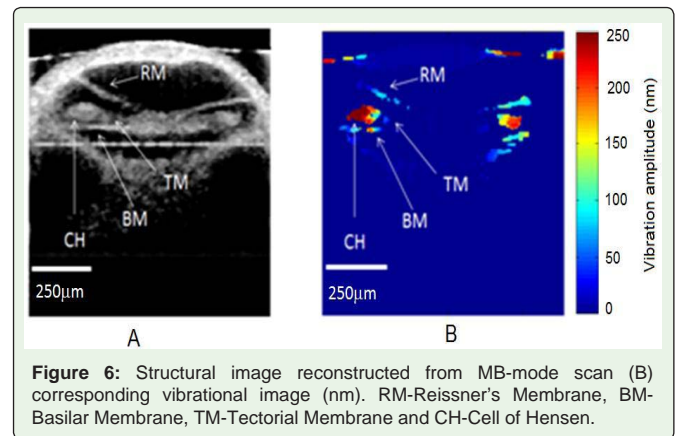

Figure 6: Structural image reconstructed from MB-mode scan (B) corresponding vibrational image (nm). RM-Reissner's Membrane, BM-Basilar Membrane, TM-Tectorial Membrane and CH-Cell of Hensen.

Hensen (CH) under acoustic stimulation condition. The magnitude of the detected vibration is indicated in the color map. In contrast, no vibrations were detected from static structures such as the otic capsule bone and the modiolus.

Summary

In summary, we have demonstrated the feasibility of using catoptrics based objective for obtaining high sensitive structural and functional imaging of cochlear microstructures through intact bone. The proposed system has several advantages. It has no refracting elements in the sample arm, or optical cement, and has no chromatic aberrations. The catoptrics configuration can provide relatively long working distance, large NA and support a broad band of optical spectrum with very high collection efficiency for back reflected light compared to conventional refractive microscope objectives. The use of high NA restricts the axial field of view to few hundreds of microns in highly scattering tissue, which is smaller than the axial field of view of traditional OCT with relatively low NA. However, the central obscuration of catoptric can also provide genuine benefit of annular apodization, which can allow enhanced lateral resolution and uphold this resolution over an extended focal depth [14]. We believe catoptric objective based OCT may provide a powerful approach for structural and functional imaging of near tissue subsurface in the optical coherence microscopy regime with enhanced sensitivity.

Acknowledgement

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