1. Specific Aims

Specific aims for this work can be divided into two parts

1. Development and validation of micro optical coherence tomography technique capable of imaging cochlea at 1x2x2 micron resolution in three dimensions in real time.
2. Imaging of cochlea *in vivo* in guinea pig to understand cellular mechanisms of sensorineural hearing loss.

2. Background and significance

Sensorineural hearing loss (SNHL) is the most common sensory defect in the world, which substantially degrades quality of life. SNHL is primarily caused by defects or damage to the cochlea of the inner ear. Damage to cochlea can be in form of a physiological damage or degenerative damage to the sensory hair cells, within the organ of Corti, neurons and other cochlear cell types (1). Sensory cells are organized into a row of inner hair cells (IHC) and 3 rows of outer hair cells (OHC), supported by pillar, Deiters, and Hensen's cells (1). When activated by sound, the inner hair cells transduce mechanical vibration into neurotransmitters that simulates neurites of spiral ganglion neurons. This electric signal travels through central auditory nuclei all the way to the cortex. Besides IHC and OHC there are other important microstructures such as the basilar membrane and Reissner's membrane whose structural integrity is critical in maintaining the functioning of ear.

Diagnosis of SNHL requires information on such microstructures within cochlea. However, complex cochlea anatomy makes it a challenging task to study these microstructures.Traditionally, such structures have been studied via histology, which is a time consuming and destructive process. Such a process does not allow studying cochlea *in vivo* and progression of diseases over time. For *in vivo*studies, techniques such as magnetic resonance imaging (2) and computed tomography (2) have been used but these techniques suffer from poor spatial resolution in the range of 0.5 mm and 1 mm respectively. Because of the limited spatial resolutions, these techniques can only detect gross abnormalities in the cochlea. Recently high resolution techniques such as optical coherence tomography (OCT) (3)and high frequency ultrasound biomicroscopy (4) have been applied to cochlear imaging providing resolution in the range of 10 microns and 30 microns respectively. Resolution provided by these techniques is still not sufficient to resolve the anatomical features such as inner hair cells, outer hair cells, supporting cells, and nerve fibers. Therefore there is great need for a technique which can images these microstructures *in vivo*.

Over the years, our lab has developed a variant of optical coherence tomography, we call micro optical coherence tomography (μOCT) (5) which provides a micron resolution in axial direction and 2 micron spatial resolution. Benchtop μOCT was utilized to detect cholesterol crystals within macrophages in atherosclerosis (5), to visualize functional anatomy (6), including individual beating cilia involved in mucociliary clearance and transport in airway epithelium (7), and to resolve cellular details in zebrafish larvae *in vivo* (8). Motivated by these results, we recently applied μOCT to study anatomy of cochlea *in situ* (9). However, application of μOCT to study cochlea *in vivo* still poses several challenges. The aim of this study is to develop a μOCT system capable of studying microstructures within cochlea *in vivo* at cellular level.

3. Preliminary Data

μOCT offers several advantages over standard OCT and other imaging modalities. μOCT utilizes infra-red and near infra-red light sources which are safer than light sources at shorter wavelength. μOCT and OCT in general do not require any contrast agent to image the sample structures and the images can be displayed in real time. In our lab we have developed a bench top μOCT system which is capable of visualizing the microstructures in three dimensions at 1x2x2 micron resolution (5). The details of the system are discussed further.

Our bench top system is based on spectral-domain optical coherence tomography (SD-OCT), described in our previous publication (5). A schematic of the bench top system is shown in Figure 1. Briefly, light from a broadband supercontinuum light source (after transmitting through a beam splitter (BS), was coupled to a single mode fiber using lens (L). Light from the single mode fiber was then collimated using another lens. The central portion of the collimated light was reflected using a 45 degree angled apodizing mirror (AM) and used as reference light. The reference light was focused using a lens (focal length=18 mm) onto the reference mirror (RM), which was placed on a translational stage. The light transmitted around the rod mirror was focused onto the sample using a lens (focal length=18 mm). The use of an apodized sample arm beam increased the depth of focus in comparison to that obtained when using a full aperture (5). The focused sample arm light was scanned over the sample using a galvanometer (GM)-based scanner, driven by sawtooth wave. The reflected reference and sample light were combined back at the apodizing mirror beam splitter and coupled back into single mode fiber. A 50/50 beam splitter (BS) reflect part of the light from the single mode fiber towards a spectrometer which consisted of a 900 lines per mm dispersion grating (Wasatch Photonics, USA), 80 mm focal length multi-element lens (Nikon, Japan), and a 4096-pixel line scan camera (Sprint spL4096Basler-140k, Basler AG). The spectrometer recorded the wavelength-dependent interference signal, which was then transferred to a computer using a camera link (CL) cable to a frame grabber (Karbon-CL4-SP, BitFlow), and processed using custom-designed Labview (National Instruments) software. Only wavelengths from 625 nm to 911 nm contributed to the usable spectrum that had a full width half maxima (FWHM) bandwidth of 140 nm. This spectrum provided a FWHM theoretical axial resolution of 1.85 µm in air.

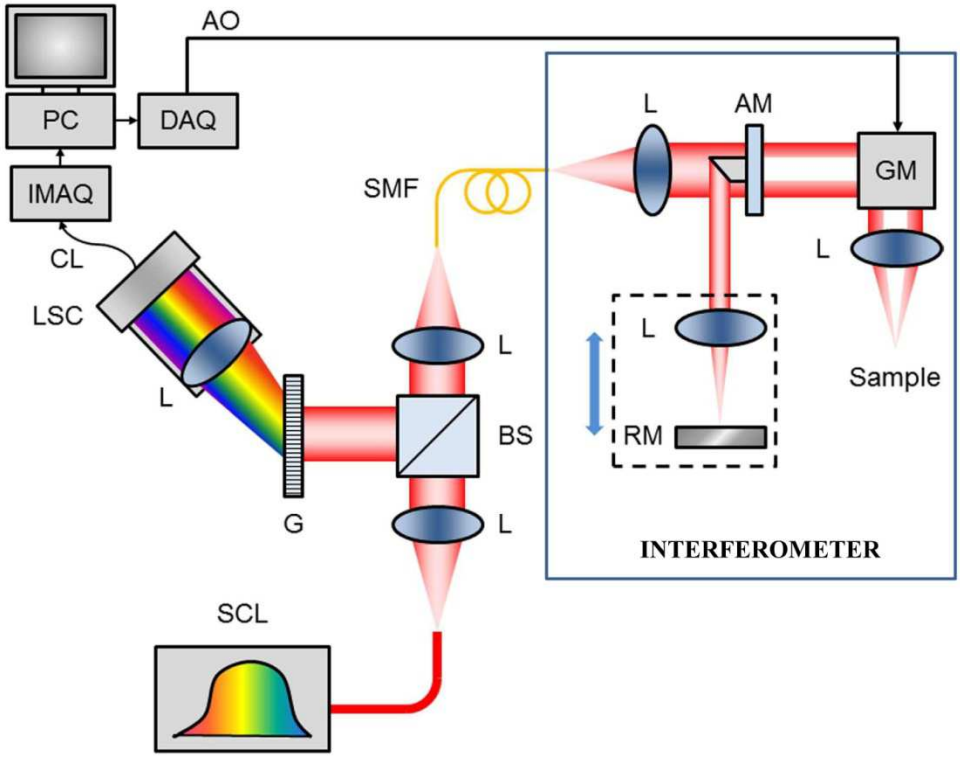

Figure 1.Schematic of the bench-top spectral domain optical coherence tomography system.

To demonstrate the potential of µOCT in cochlea imaging we utilized our bench top system to image guinea pig cochlea *in situ*. In Figure 2 we show the preliminary results on cochlea imaging with bench top µOCT. Figure 2 (**a**) is volumetric reconstruction of the µOCT image where nerve fiber bundles traversing the organ of Corti towards the outer hair cell region can be seen. Top right corner presents the 3D modal of the cochlea and a plane transversing cochlea representing the location of the image within cochlea. Scale =150 µm. Figure 2 (**b**) is schematic representation of the features observed in Figure 2 (**a**), where bundles of nerve fibers (NF) can be seen crossing the tunnel ofCorti (TC) and/or the space of Nuel (SN). Outer pillar cells (OPC) can also be identified. Scale= 150 µm. To compare µOCT images with images from other modalities we also acquired images of the guinea pig organ of Corti with a confocal laser scanning microscope which is shown in Figure 2 (**c**). Rhodaminephalloidin (red) marks outer and inner

pillar cells (OPC and IPC, respectively), Hoechst stain (blue) marks cell nuclei, and neurofilament-H (green) marks neuronal fibers Scale = 50 μm.

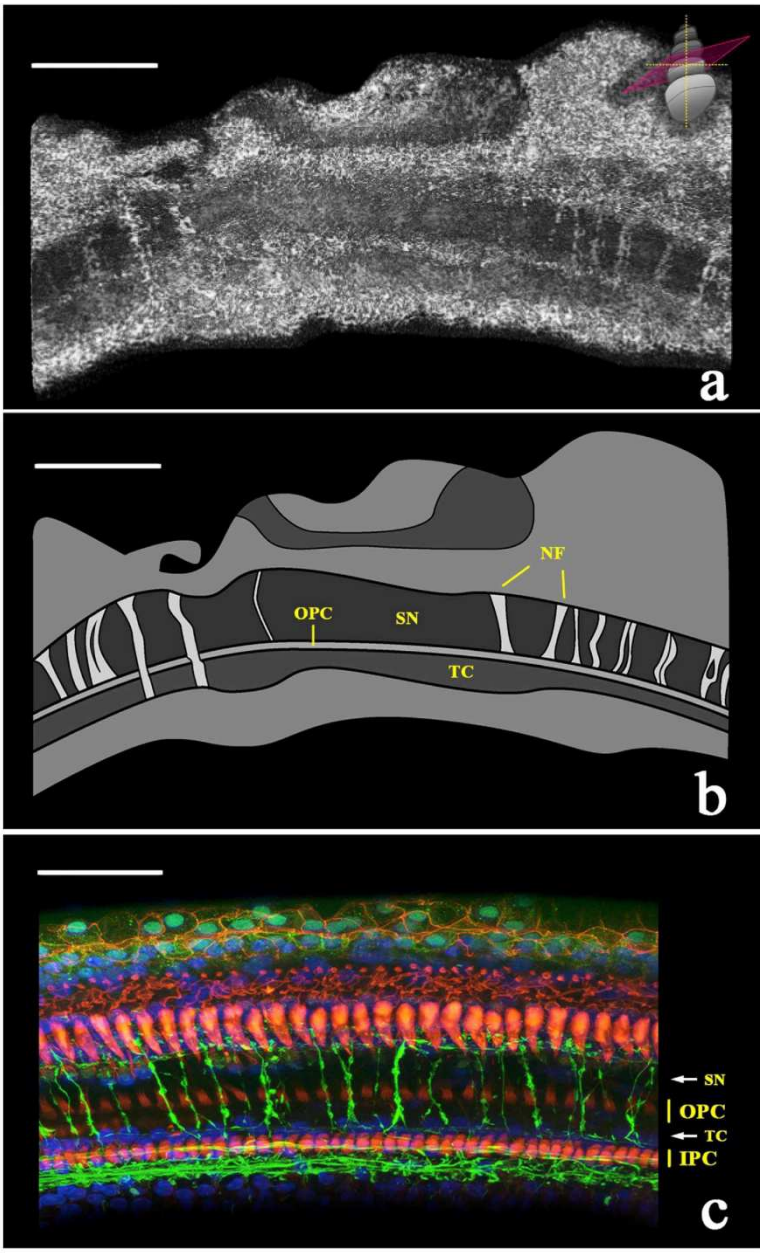

Figure 2.Images of nerve fiber bundles traversing the tunnel of Corti and space of Nuel to innervate outer hair cells.

4. Experimental design and methods

The result with bench top system demonstrated μOCT's potential to image cochlea organ and motivates further miniaturization of μOCT technology. However, significant development is required before this technology can be employed to assess cochlear pathology in living humans. Our lab recently developed miniaturized probes for airway cilia imaging *in vivo* (7) and probes suitable coronary artery imaging (10). However, probe development for cochlea imaging remains a significant technical challenge due to constraints of the small size and embeddedlocation of the human cochlea.

Cochlea being an organ with conical spiral shape requires a flexible probe to image it. The diameter of cochlea can vary anywhere from few millimeter to few hundred microns. This limits the probe diameter to few hundred microns only. Bench top μOCT provides 1x2x2 micron resolution in three dimensions with depth of focus of 300 microns. This depth of focus is not sufficient to image cochlea *in vivo* because of the variation in the circumference which can range from few millimeters to few hundred microns. With these constraints in mind, I have designed an ultra miniaturized probe whose schematic is shown in Figure 3. This probe will replace the interferometer part of the bench top μOCT system shown in Figure 1. The probe is based on novel design recently reported by our lab in which a multimode fiber is used as mirror tunnel. A single mode fiber (SMF) is inserted into a drive shaft which provides mechanical stability to fragile fiber. Drive shaft will also translate the necessary torque required to rotate the probe during scanning. Outer diameter of the drive shaft will be 550 microns which similar to drive shaft used in standard coronary artery imaging probe. Drive shaft will be enclosed in an 800 micron diameter sheath. At the distal end, sheath will be tapered down to an outer diameter of 400 microns and inner diameter of 250 microns. This step is necessary since 800 micron diameter probe would not reach deeper into the

cochlea. Beyond the tapering point there will be no drive shaft but only bare single mode fiber (SMF) with focusing optics.

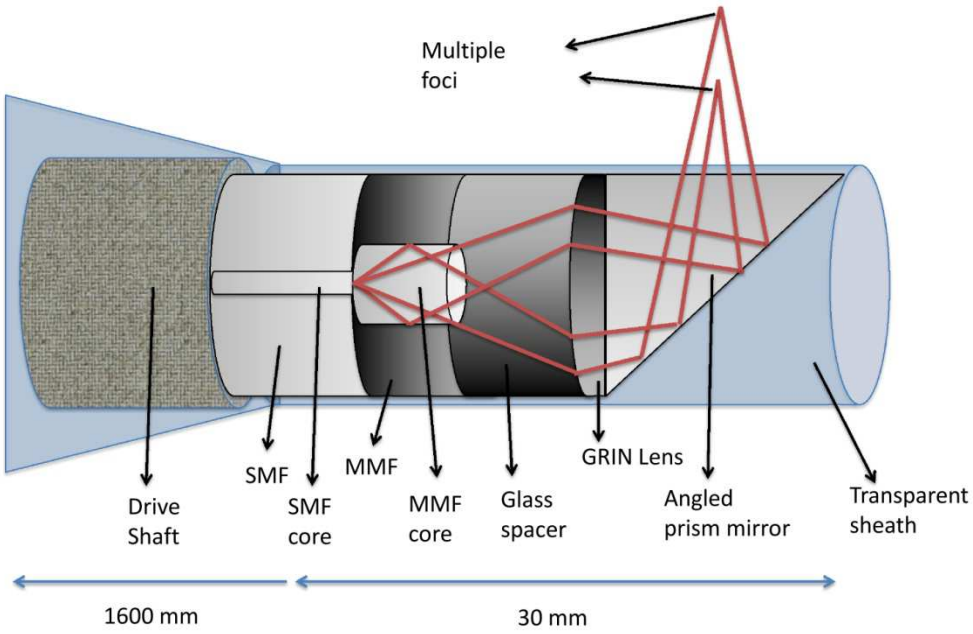

Figure 3. Schematic of the proposed design for cochlea imaging probe

A multimode fiber (MMF) with core diameter 62.5 micron will be spliced at the end of SMF. Light out of SMF core will excite multimode in the multimode fiber section. A glass spacer will be spliced to MMF fiber tip so that beam can expand within this section providing enough numerical aperture for high resolution imaging. After glass spacer a GRIN fiber will be spliced to focus the modes excited within MMF. An angled prism mirror will be attached at the end of the GRIN fiber to deflect the beam onto the side to make it a side viewing probe. The beam will exit the sheath and focus on cochlea tissue. A 400 micron sheath will cause strong astigmatic effects and such effects can be avoided by using a curved reflector instead of flat reflector. I will perform optical simulations using Zemax optical studio software to determine the necessary optics for correcting the sheath astigmatism. A bare fiber is very fragile and to reduce the risk of damage during operation, the probe assembly after tapering point will be polyamide coated providing stability to the fiber assembly.

The performance of the designed probe was simulated using Zemax optical studio software and simulated results are shown in Figure 4. Along the x- axis is ploted the distance from the probe tip and along y-axis the diameter of the focused spot. The simulated results suggest that the designed probe will produce multiple focal points along the axial direction. Full width half maxima of the focal spots between 0.5 mm and 1.7 mm from the tip of the probe will be approximately 2 microns giving us a depth of focus of 1.2 mm which is approximately 4 times higher than the bench top system while maintaining similar axial and spatial resolution.

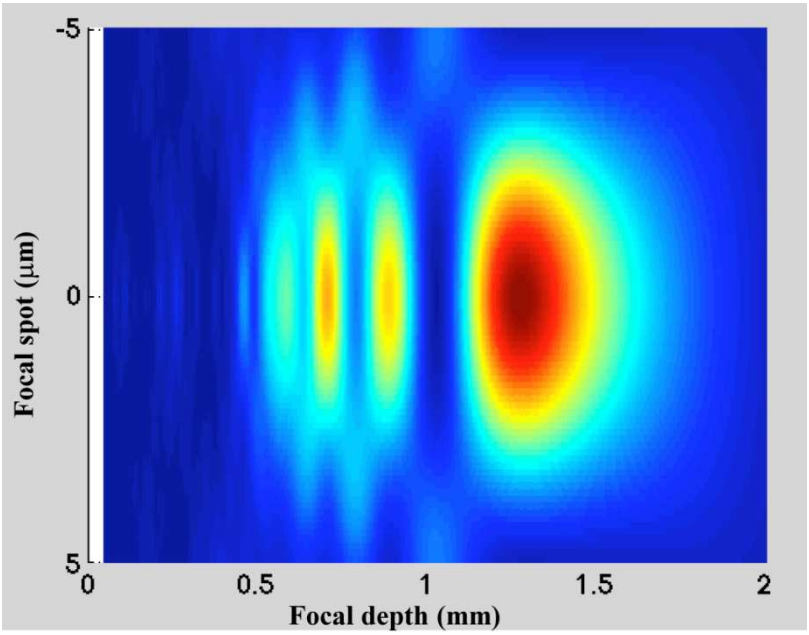

Figure 4. Simulated results for focal properties of the designed probe.

I fabricated a prototype probe based on the proposed design and results obtained were in good

agreement with the simulated results. The tests were done however without sheath which will cause significant artifacts due to small curvature. In near future, I will perform simulations to determine the correct curvature of the reflecting prism so that the curvature of the sheath can be compensated.

In vivo imaging of cochlea in guinea pig (AIM 2)

After successful fabrication of the final probe, I will utilize the probe to perform in vivo imaging of cochlea in guinea pig. For this we will procure 40 guinea pigs and perform experiments at our collaborators facility at Massachusetts Eye and Ear Infirmary, Boston. We have already obtained the approval from Institutional Review Board at Massachusetts General Hospital and have an active protocol to perform the experiments. After the μOCT imaging session, animal will be sacrificed and cochlea will be surgically removed by trained personnel for histopathology analysis. The images acquired from μOCT will be compared against gold standard histology images.

5. References

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6. Potential scientific and medical impact

Expertise of my mentors Dr Gary Tearney in development of high resolution imaging techniques and Dr Stankovic in cochlearanatomy and pathology guarantees the success of this project which will add to the improvement of quality of life in general population and especially in older people and newborns where senosirnuearl hearing loss is the most common congenital anomaly, affecting 1:500 newborns.. This will also contribute greatly to the advancement of knowledge since at present there is no technique which can image cochlea at micron level resolution *in vivo*. The preliminary results obtained from simulation and experiments are very promising and meet the desired specification for this study. Further improvements and research is however required to perform experiments *in vivo*.