Dual-mode endoscopic probe combining OCT and autofluorescence imaging for inner ear hearing loss diagnosis and therapy guidance

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Outline

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- **Introduction**
  - Sensorineural Hearing Loss (SNHL) and its diagnosis and treatment
  - Optical coherence tomography (OCT) and Auto- fluorescence (AF)
- **Methods**
  - Research approach:
    - Proof of Concept - Bench-top OCT/AF imager (completed)
    - Preliminary Study - Endoscopic OCT/AF probe instrument (in progress)
  - Experimental setups in Proof of Concept
- **Results**
  - Results in Proof of Concept
  - Current Status in Preliminary Study
- **Future Plan**
- **Discussion and Conclusion**

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Sensorineural Hearing Loss (SNHL)

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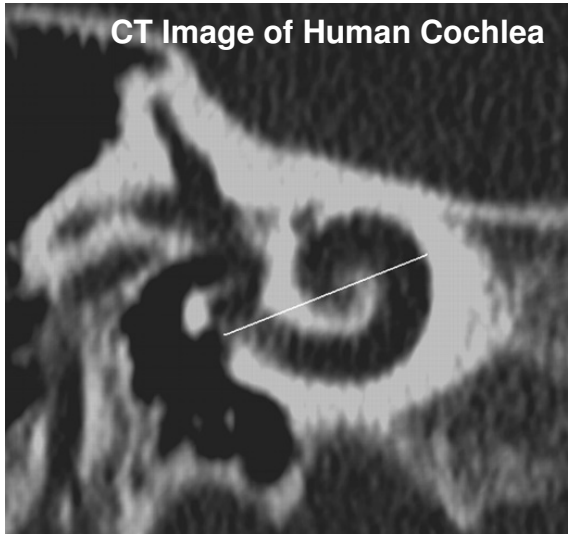
- **Sensorineural Hearing Loss (SNHL)**
  - Progressive or sudden damage of either the inner ear or the nerve between the inner ear and the brain.
  - About 90% of reported hearing loss (over 200,000/year in US)
- **Diagnosis of SNHL**
  - Physical examination (e.g. Otoscopy)
  - Simple differential testing (e.g. Weber testing, Rinne testing)
  - Complex auditory function testing (e.g. oto-acoustic emissions, acoustic reflexes, speech audiometry and evoked response audiometry)
  - Imaging approach (e.g. CT and MRI)



- **Treatment of SNHL**
  - Traditional treatment: Hearing aids and Cochlear implant
  - New treatment: Drug delivery and Stem cell/gene therapy by regenerating inner ear hair cells and cochlear neurons

Current Limitation of SNHL Diagnosis/Treatment

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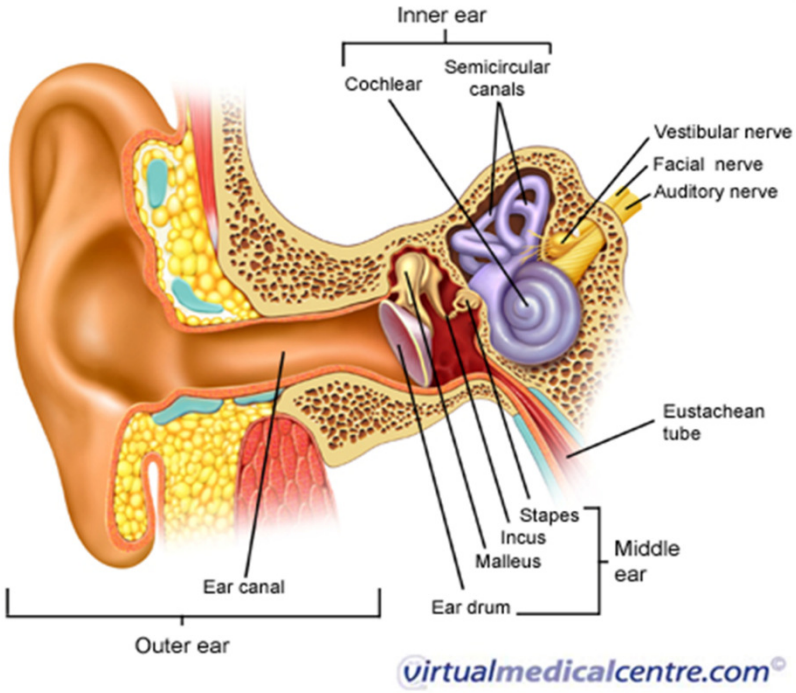
- **The diagnosis/treatment of SNHL is impacted by:**
  - Difficulty of accessibility to cochlea
  - Inability to visualize tissue damage at the cellular level
  - Complex mechanism of hearing loss with morphology and functionality.
  - No direct approach to evaluate the new treatment such as hair cell regeneration



Goal: Develop an dual-modal endoscopic optical imaging instrument by providing minimally-invasive and real-time evaluation of cochlear morphology and functionality to improve the SNHL diagnosis and therapy guidance

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Anatomy of Cochlea

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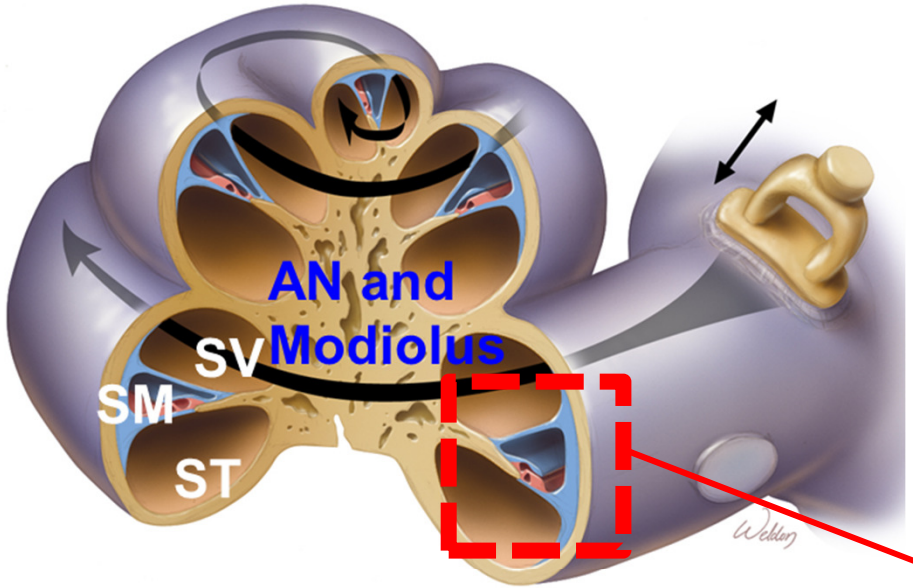
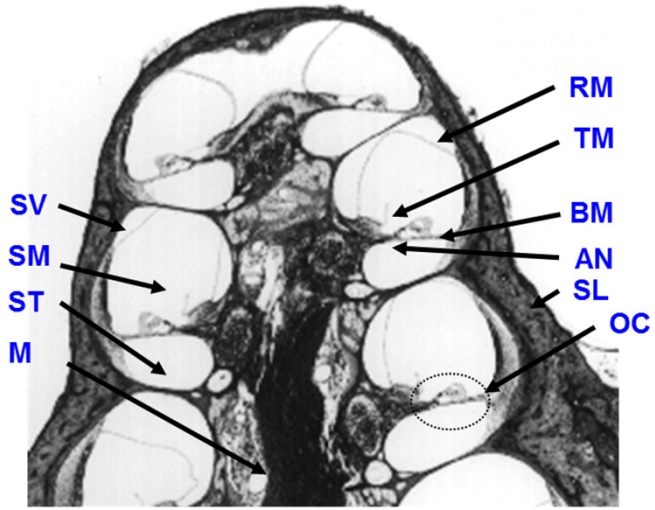
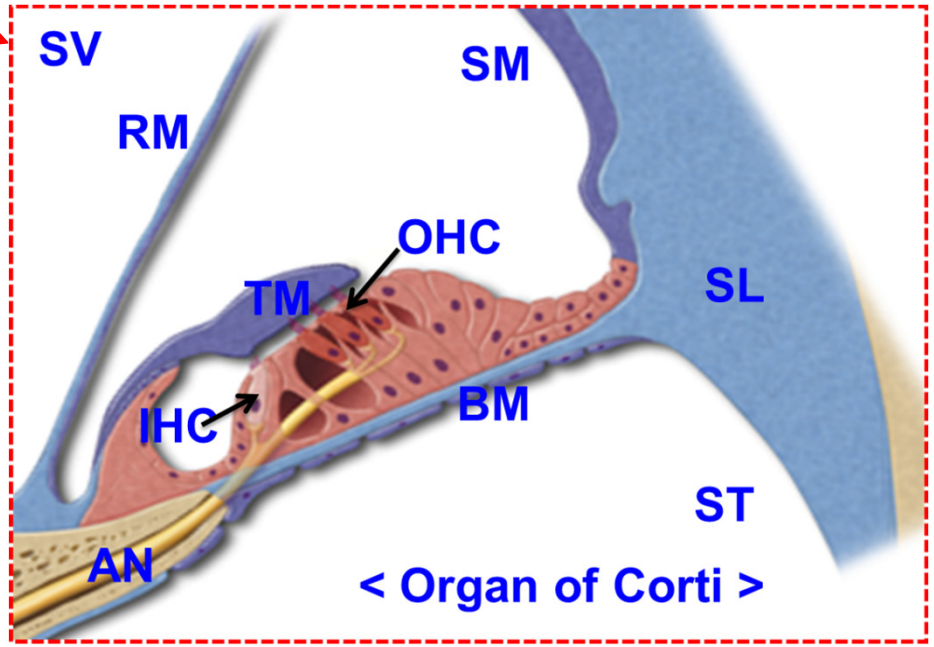


Auditory nerve (AN)
Scala vestibuli (SV) or Vestibular Duct
Scala media (SM) or Cochlear Duct
Scala tympani (ST) or Tympanic Duct
Reissner's membrane (RM)
Tectorial membrane (TM)
Basilar membrane (BM)
Auditory nerve (AN)
Spiral ligament (SL)
Inner hair cell (IHC)
Outer hair cell (OHC)

Cross-Section View



Micrograph from http://oto2.wustl.edu



Modified from https://spie.org/news/5504-observing-cochlear-function?SSO=1

OCT/AF combined use Benefits

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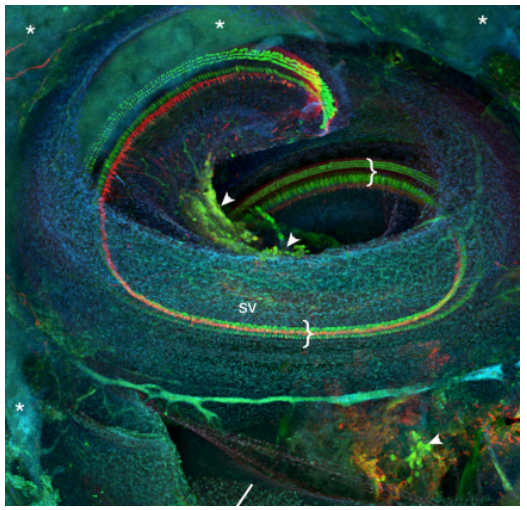
Complement each other:

Optical Coherence Tomography (OCT) can visualizes morphological disruption or deformation of organ of Corti,

Single-Photon Autofluorescence imaging (AFI) can detect loss of hair cell functionality- reduced metabolism and flavoprotein of hair cells in the basal synaptic region

1310 nm OCT
https://med.stanford.edu/

OCT ⟷ FI



Two-photon fluorescence
http://depts.washington.edu/

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Research Approach

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- **Proof of concept (completed)**
  - Aims: (1) Develop a dual-modal OCT/AF imager
    (2) Perform the testing of the OCT/AF imager in a normal and hearing-impaired rodent model *ex vivo* and *in vivo*
  - System: *Bench-top OCT/AF imager*
  - Experiment:
    - Animal testing: *Ex-vivo and in-vivo* normal vs. hearing-impaired mice
- **Preclinical Study (in progress)**
  - Aims: (1) Develop a dual-modal endoscopic OCT/AF probe system
    (2) Validate the OCT/AF endoscopic imaging on both *ex vivo* cadaveric ear and *in vivo* ovine animal model of hearing loss
  - System: *Endoscopic OCT/AF probe instrument*
  - Experiment:
    - Validation testing: Cochlear Phantom, and *Ex-vivo* human cadaveric and ovine temporal bone
    - Animal testing: *In-vivo* cochlear imaging of normal and hearing-impaired ovine model

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Proof of concept: Bench-top OCT/AF imager

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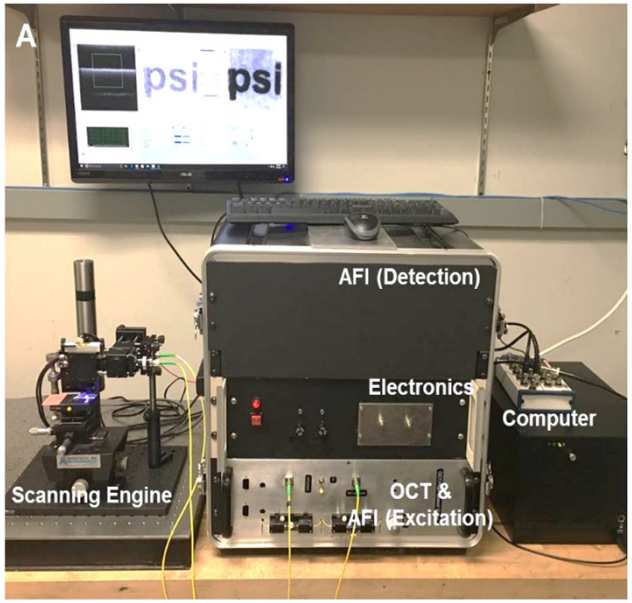
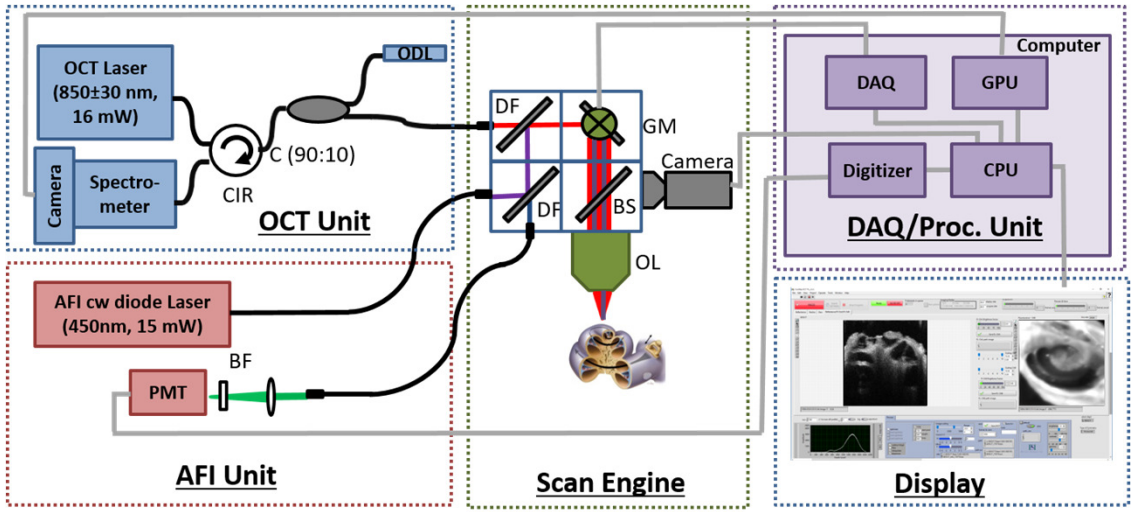
- **OCT/AF Imager :** 1) OCT Unit, 2) AF unit, 3) Scan engine, 4) Data Acquisition and Processing Unit, and 5) Display
- **Benefits/limitations**
  - Improves power management
  - Cumbersome- Open-air system
  - Not applicable for pre- and clinical study





Beam Splitter (BS), Circulator (CIR), Coupler (C), Dichroic longpass filter (DF), Optical delay line (ODL), Galvanometer-controlled mirror (GM), Objective lens (OL), Bandpass filters (BF), and Photomultiplier tube (PMT)

Design Parameters (Bench-top OCT/AF Imager)	
OCT wavelength	850 ± 30nm
A-line Speed	60 kHz
Imaging range-axial	1.5 mm
Axial resolution	7.5 µm
Lateral resolution	10 µm
Field of View	3 mm
Fluorophore	FAD
Ex. wavelength	450 nm
Em. wavelength	525 nm ± 25nm

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Proof of Concept: Experiment

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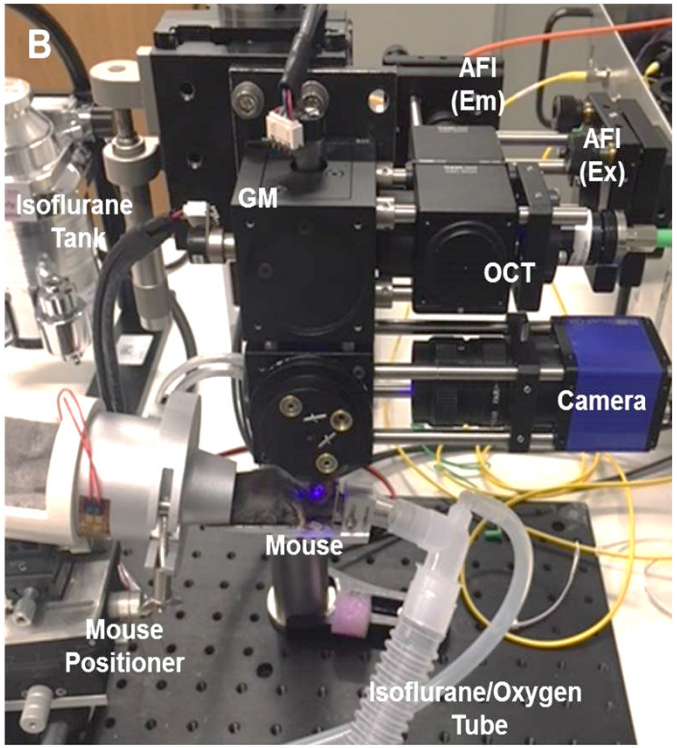
- **Animals**
  - Protocol Under the approval from PSI IACUC boards
  - Normal CFW and Usher1c216(G>A) mutant mice
- **Preliminary experiment with normal excised cochlea**
  - Sacrifice of mice and surgical removal of cochlea
  - OCT and AF imaging were performed
- ***Ex-vivo* experiment with normal and hearing impaired cochleae**
  - OCT and AF imaging were performed within 10 min.
  - Immunofluorence imaging of the cochlea was performed to detect surviving hair cells with Alexa Fluor 488.



- ***In-vivo* Experiment**
  - Moderately anesthetized with isoflurane/oxygen
  - Surgery was performed to expose the cochlea under a dissecting microscope.
  - Anesthetized mouse was placed on a customized mouse positioner.
  - OCT volumetric scans and AF *en-face* scans were performed simultaneously.

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Proof of Concept: Preliminary OCT/AF Images

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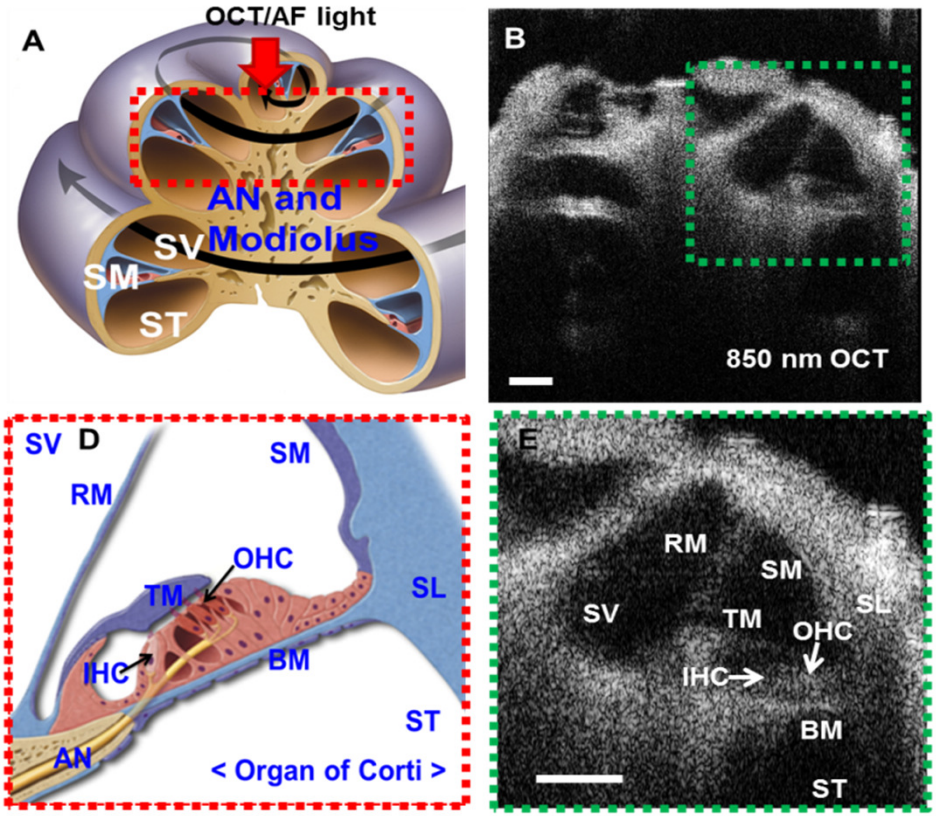
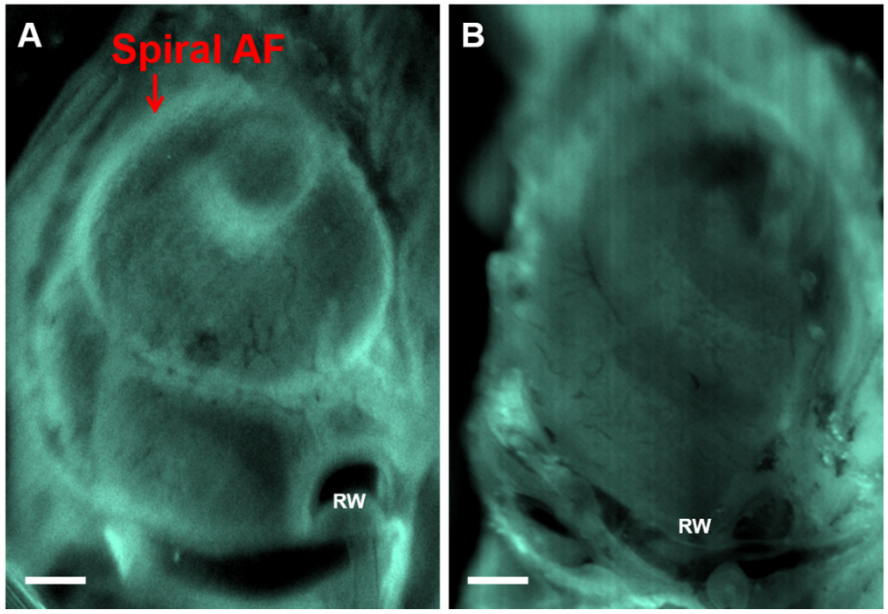
- **Ex-vivo normal mouse cochlea**

OCT Cross-Section Image

AF Images at different time period





~ 10 min after excision

~ 24 hr after excision

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Auditory nerve (AN), Scala vestibuli (SV), Scala media (SM), Scala tympani (ST), Reissner's membrane (RM), Tectorial membrane (TM), Basilar membrane (BM), Spiral ligament (SL), Inner hair cell (IHC), and Outer hair cell (OHC). The scale bar is 200 µm.

Proof of Concept: Ex-vivo OCT/AF Images

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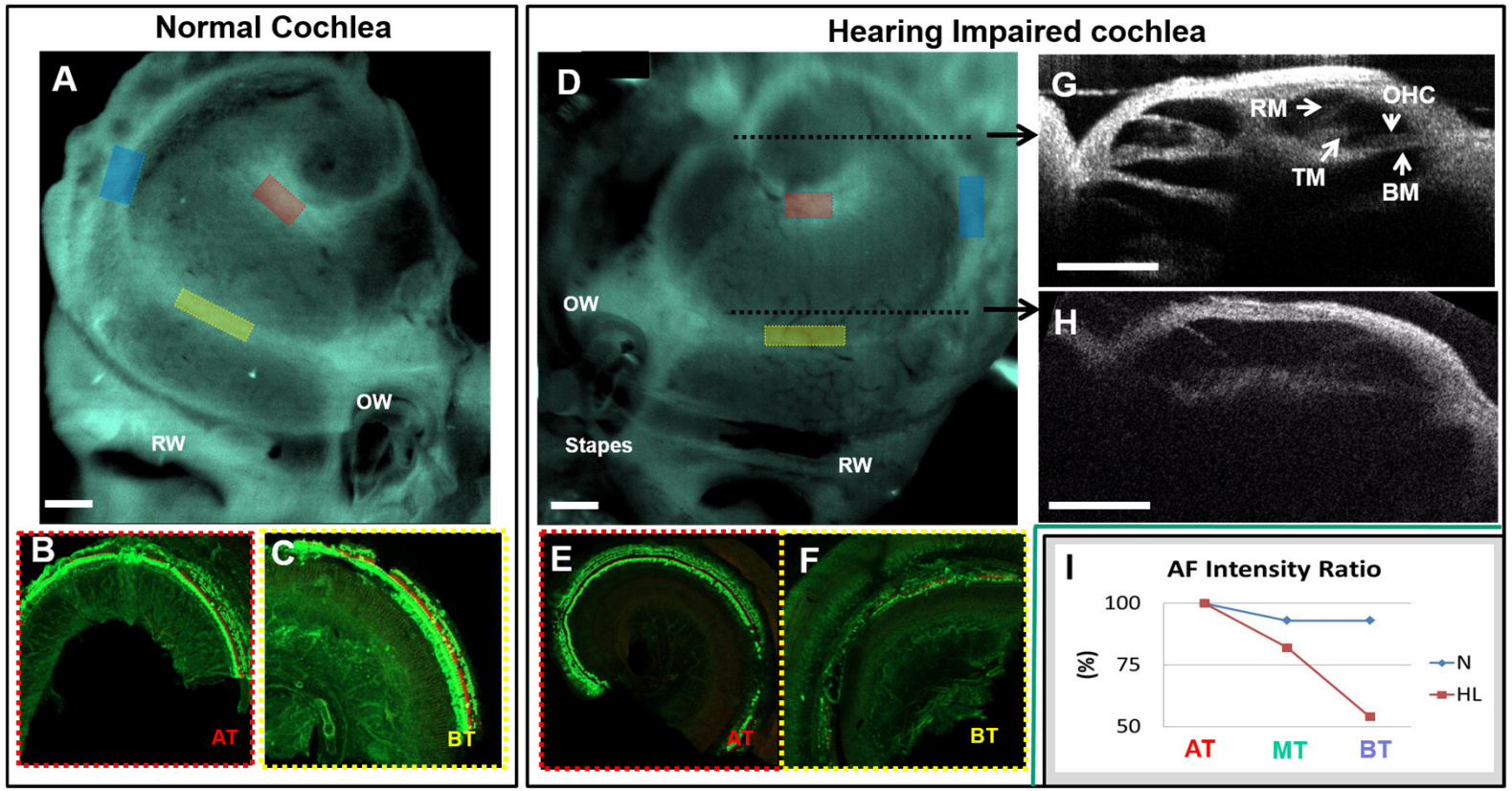
- **Ex-vivo normal vs. hearing-impaired mouse cochleae and associated Immuno-fluorescent image**



Apical turn (AT), Middle turn (MT), Basal turn (BT), Reissner's membrane (RM), Tectorial membrane (TM), Basilar membrane (BM), Outer hair cell (OHC), Oval window (OW), Round Window (RW), Normal (N), and Hearing loss (HL). Scale bar = 200 µm

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Proof of Concept: In-Vivo OCT/AF Images

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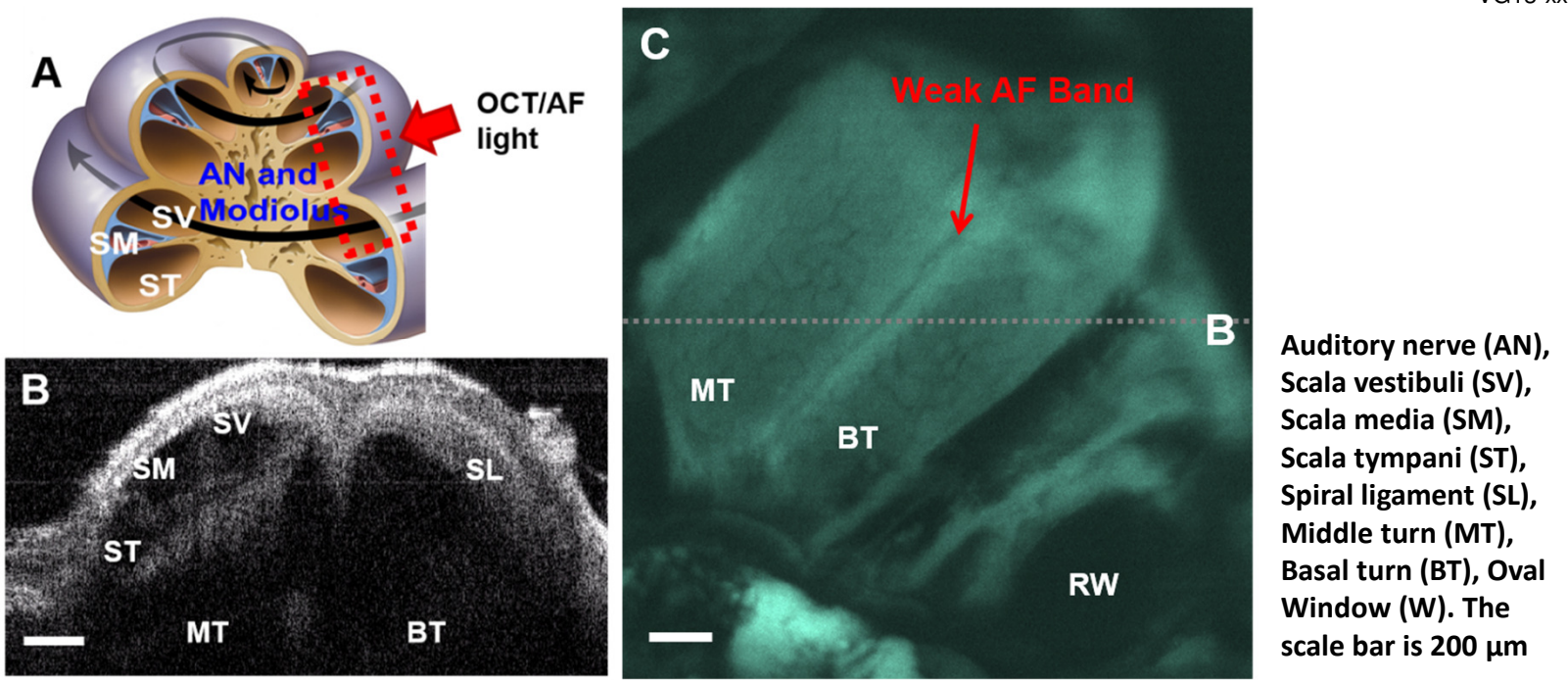

Auditory nerve (AN), Scala vestibuli (SV), Scala media (SM), Scala tympani (ST), Spiral ligament (SL), Middle turn (MT), Basal turn (BT), Oval Window (W). The scale bar is 200 µm

- **Difficulties of in-vivo cochlear imaging using the bench-top OCT/AF imager**
  - *The deep and steep angled position of the cochlea* surrounded by the muscle, blood vessels, and adipose tissues.
  - *Thick spiral ligament and cochlear bone* in middle and basal turns
  - *The imaging position of Organ of Corti* Including hair cells are perpendicular to OCT/AF light beam
  - **Endoscopic probe based imaging should overcome these issues !!**

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Preclinical Study: Endoscopic OCT/AF probe instrument

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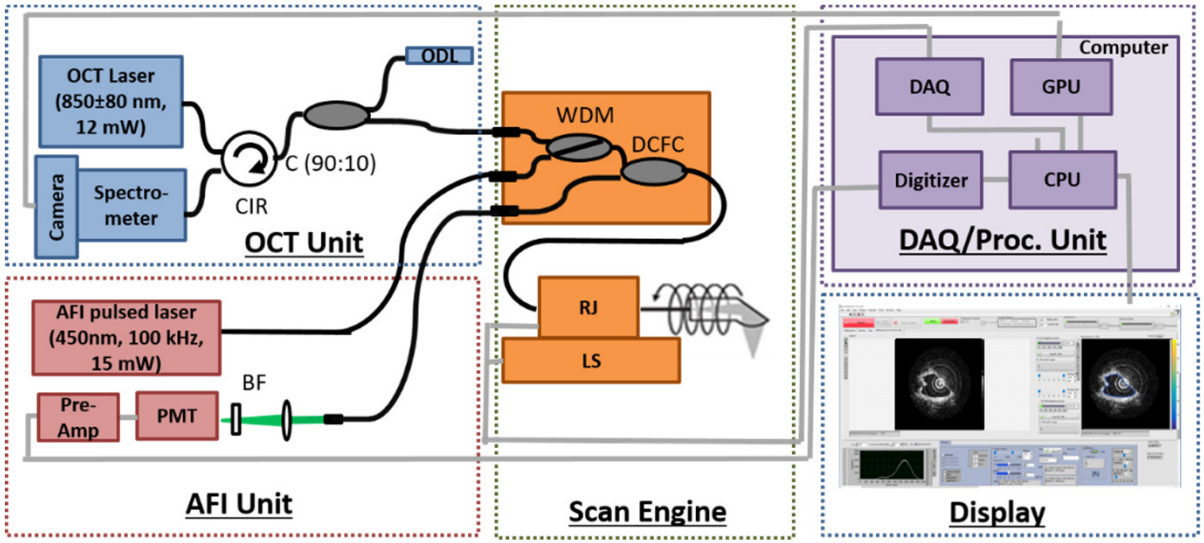
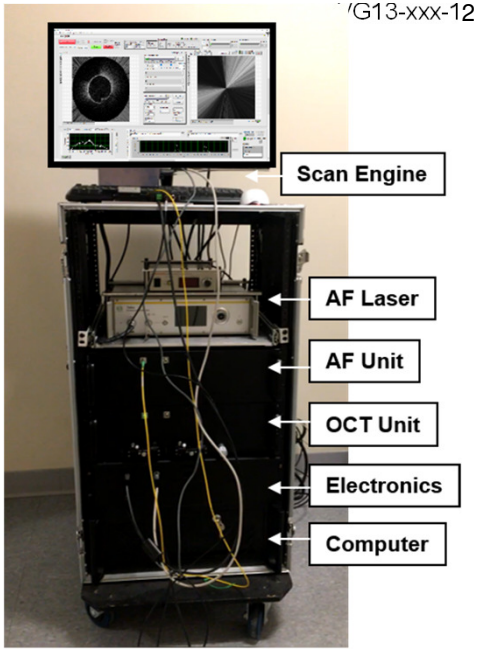
- **Upgrade fiber-based endoscopic OCT/AF probe instrument :**
  - **OCT Unit**: Improved bandwidth, axial resolution, imaging depth, and A-line speed
  - **AF unit**: Enhanced florescence detection using single photon-counting approach using TCSPC board
  - **Scan Engine**: Developed dual-mode endoscopic probe with a WDM/DCFC module and circumferential scanning with rotary junction and linear motor
  - **Data Acq. and Proc. Unit**: 3D circumferential scan and control
  - **Display**: Overlaid display of both OCT/AF modes



Wavelength division multiplexer (WDM), Double-clad fiber coupler (DCFC)



Design Parameters (Endoscopic OCT/AF Probe)	
OCT wavelength	850 ± 80 nm
A-line Speed	250 kHz
Imaging depth	2.0 mm
Axial resolution	4.0 µm
Lateral resolution	10-15 µm
Field of View	360 deg
Fluorophore	FAD
Ex. wavelength	450 nm
Em. wavelength	525 ± 25 nm

Preclinical Study: Scan Engine and OCT/AF probe

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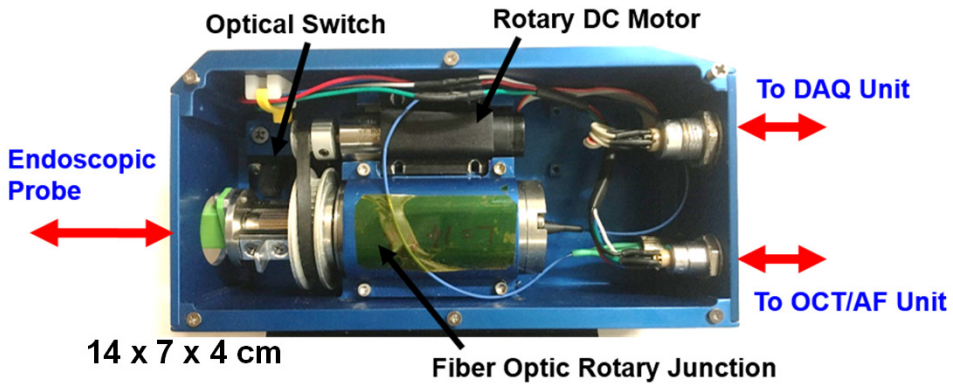
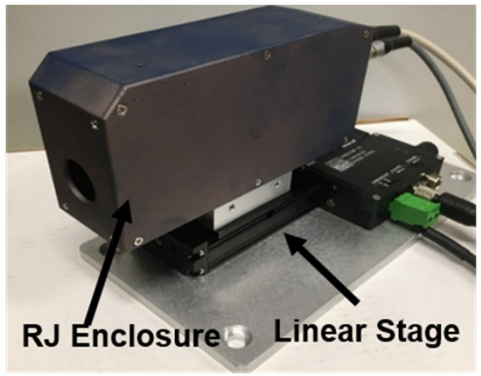
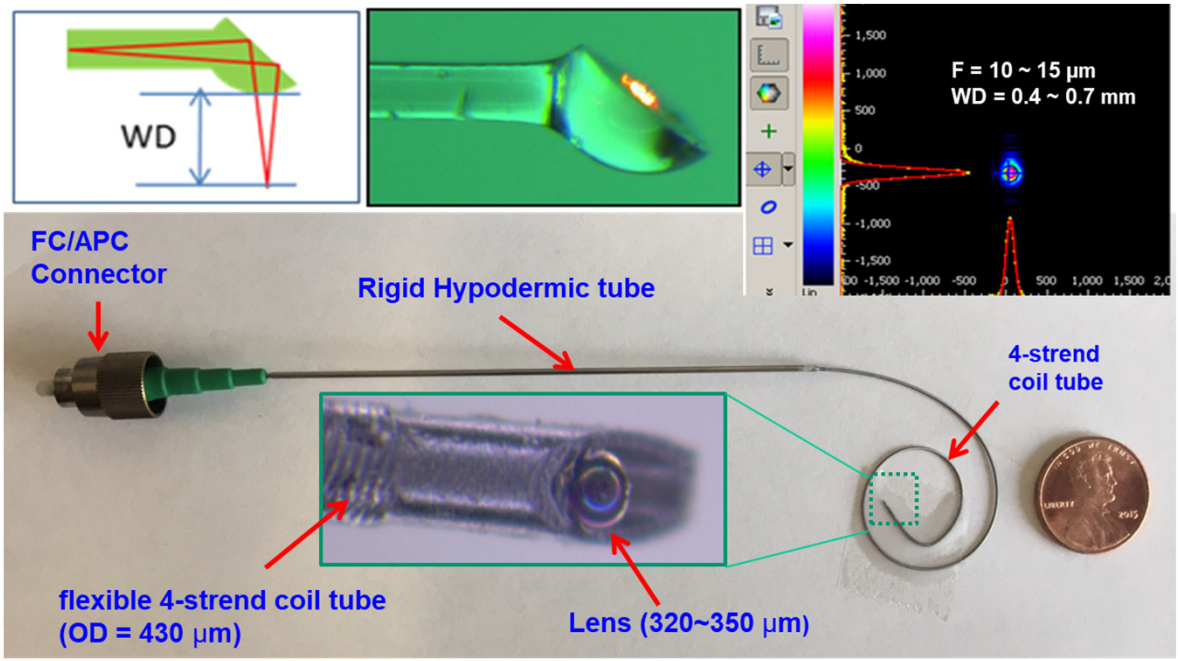
- **Scan Engine (Rotary Junction and Linear stage)**





- **Ball-lens based endoscopic probe (Prototype)**



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Preclinical Study: Cochlear Phantom and Insertion Test

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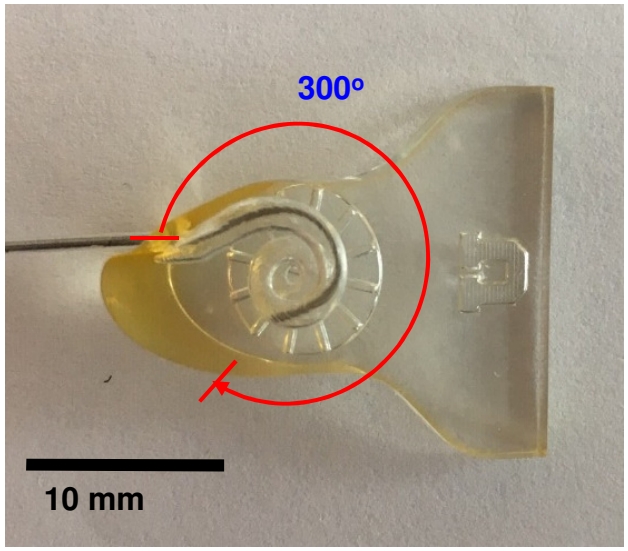
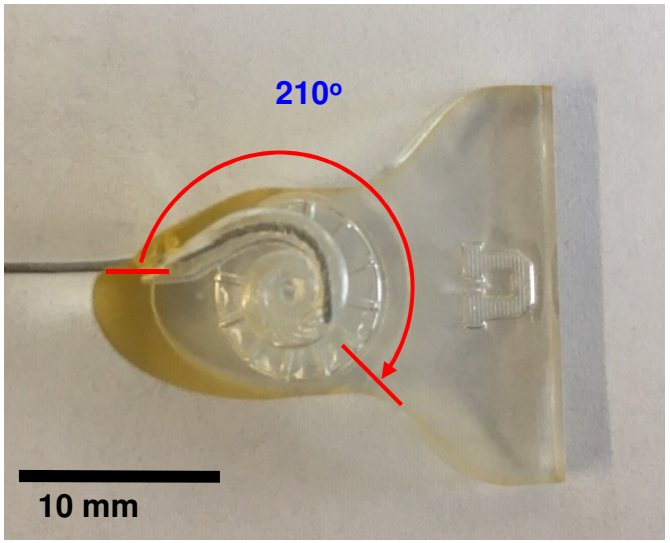
- **3D printed Cochlear Phantom**
  - Resource: *J. Med. Devices*. Dec 2014, 8(4): 041010 (https://www.telerobotics.utah.edu/index.php/Research/CochlearImplants)

- **Endoscopic probe Insertion Testing**
  - Insertion of the probe through a round window (Diameter 2.3 x 1.87 mm in Human)



Maximum insertion (300° of first turn, ~ 20 mm long)



Optimal insertion (210° of first turn, ~ 15 mm long)

Preclinical Study: Future Plans

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- **Finalize the fabrication of the endoscopic OCT/AF probe instrument**
  - Assemble the double-clad fiber optic rotary junction
  - Build the endoscopic probes (e.g encapsulate the protection tubes and connectorize probe)
- **Optimize endoscopic OCT/AF probe on *Cochlear phantom***
  - Test the performance parameters of the instrument (e.g. axial and lateral resolution, imaging range, and AF detection efficiency)
- **Evaluate endoscopic OCT/AF probe on *ex-vivo ovine model***
  - Fresh ovine temporal bones from autopsy specimens with intact cochlear structures
  - Validate the OCT/AF imaging capability by demonstrating intracochlear structures and biochemical functionality
- **Perform endoscopic OCT/AF probe imaging on *in-vivo ovine model***
  - Normal and Noise-induced SNHL animals
  - Obtain endoscopic OCT/AF images between normal and SNHL animals
  - Analyze the endoscopic OCT/AF images for diagnose/treatment of SNHL

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Discussion and Conclusion

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- **Diagnosis and therapy evolution of SNHL with *current imaging modalities is limited due to difficulty of accessing and performing structural and functional measurement in the inner ear*.**

- **Proof of Concept study has demonstrated that the combined *OCT/AF imaging approach can detect morphological and biochemical changes related to inner ear SNHL*.**

- **Preclinical study of *an endoscopic OCT/AF instrument are developing for pre-clinical testing* on in-vivo sheep SNHL model.**

- **In conclusion, OCT/AF technology could be used to *diagnose potential causes of SNHL and evaluate the success of the hair cell regeneration therapy approaches*.**

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Acknowledgement

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NIDCD
National Institute on Deafness and Other Communication Disorders

NIH/NIDCD
(National Institute on Deafness and Other Communication Disorders)

- **Project Team members**

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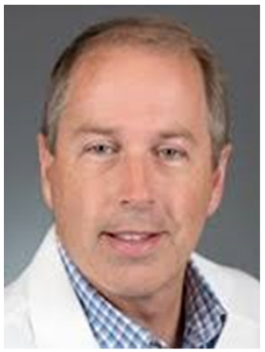

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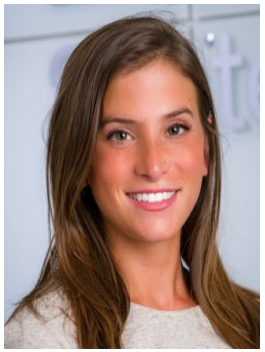

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Thank you