Fiber-optic probe and bulk-optics Spectral Domain Optical Coherence tomography systems for in vivo cochlear mechanics measurements

Nathan Ching Lin

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Abstract

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Nathan Lin

Acquiring the motions of the inner ear sensory tissues provides insight to how the cochlea works. For this purpose, Spectral Domain Optical Coherence Tomography (SDOCT) is an ideal tool as it has a penetration depth of several millimeters. SDOCT can not only image inside the cochlear partition, but also measure the sample structures’ simultaneous displacements. We customized a commercial Spectral Domain Optical Coherence Tomography system for such functions and detailed the software and hardware steps so this powerful system could be more accessible to auditory researchers.

The cochlea is surrounded by bones and tissues, and damage to it would make it passive. For this reason, cochlear vibrometry measuring locations have been limited to either the basal or apical regions. That is why I fabricated a two-dimensional scanning SDOCT-based probe, to access more cochlear locations through a small hand-drilled hole. What is exciting about the probe is that an
electrode can be attached to its side to acquire spatially and temporally coincident voltage and displacement data. This would help us better understand the cochlear mechano-electrical feedback process.

Lastly, I investigated how the SDPM-reported displacement could be influenced by its neighboring signals and demonstrated this signal competition phenomenon experimentally and theoretically.
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CHAPTER 1. Cochlear Mechanics Background

It is a wonder we can hear the loud screams at the Eagles concert, but also the buzz of a bee. Our ears can hear up to 2 Pascal (Pa) in sound pressure. Sound pressure levels (SPL) are usually represented in decibels (dB) using \( L_p (dB) = 20 \log_{10} \left( \frac{\text{sound pressure}}{20 \mu \text{Pa}} \right) \), with 0 dB being the quietest sound we can hear (referenced to 20 \( \mu \)Pa) and 100 dB being the loudest we are normally exposed to in Figure 1.1. (It can go louder of course).

\[ \text{Figure 1.1. A range of sound pressure levels (SPL) from 0 to 100 decibel (dB). With 0 dB being the quietest to 100 dB being the loudest.} \]

If our ears operate linearity, then the sensory tissues would have to move up to 5 orders of magnitude to create 100 dB SPL of sound; such large movement would most likely damage the surrounding tissues. Fortunately, there is a process called cochlear amplification that intensifies the low intensity sounds so a wide dynamic range can be heard.
1.1 How sound enters the ear

When sound enters the ear, the sound wave mechanically pushes and pulls the eardrum. The movement of the eardrum moves the middle ear ossicles: malleus, incus, and stapes (in this order). The stapes then carries this mechanical energy to the oval window (at the basal region of the cochlea) [1]. This schematic of how the sound enters from the outer ear, to the middle ear, and ultimately to the inner ear is represented in Figure 1.2.

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{sound_diagram.png}
\caption{The diagram of how sound wave enters through the outer ear and hits the eardrum in the middle ear. The ossicles (bones attached to the ear drum) then pushes and pulls on the oval window to launch a traveling wave into the cochlea (in the inner ear). \textit{Used with permission of the artist H. Nakajima}}
\end{figure}
1.2 Cochlear structure

The cochlea looks like the snail’s shell, with three channels adjacent to one another: scala vestibuli, scala tympani, and the scala media [1]. The histology image of cross-sectional cochlea is shown in Figure 1.3. The scala tympani and scala vestibuli are filled with perilymph, which is an ionic fluid. The scala media is filled with endolymph (contains a high potassium concentration) and has a positive electrical potential (80 millivolts). This potential is important for the mechano-electrical feedback process- a major component of cochlear amplification [1]. The cochlea is tonotopic, with the apical region more sensitive to the lower frequencies, and the basal region more sensitive to the higher frequencies. This is mostly due to the basilar membrane’s (BM) varying stiffness and width: widest and least stiff at the apical region, and narrowest and most stiff at the base.

Figure 1.3. This shows the anatomy of the cochlea. It is composed of three ducts: scala tympani, vestibule (shown in blue) and scala media (shown in red).
1.3 Sound traveling wave in the cochlea

The plunging motion of the stapes creates a pressure difference across the cochlear channels to move the organ of Corti in a transverse manner, and the wave travels from the basal region to the apex. This phenomenon was first noted by Georg von Bekesy with his study of the human cochlear cadaver [1]. He noted that this traveling wave, of a particular frequency, propagates from the base to the apex and reaches the maximum amplitude at the best frequency place, where it peaks and then dies down (Figure 1.4). Von Bekesy was awarded the Nobel Prize in 1961 for this discovery.

**Figure 1.4.** Georg von Bekesy observed the traveling wave in the human cadaver cochlea. He showed the traveling wave profile (of a particular frequency) reaching the maximum amplitude at the best frequency place.
The traveling wave displaces the stereocilia on the inner and outer hair cells, which opens the mechanically gated ion channels. The influx of the potassium and calcium ions from the endolymph depolarizes the cell to create a voltage signal. This is termed the mechano-electrical transduction (MET) process, as it transduces the mechanical sound waves into electrical signal [1].

1.4 Hair cells and the Mechano-electrical process

For the outer hair cells, the voltage changes lead to mechanical forces that amplify the motions through a positive mechano-electrical feedback loop. The analogy is like a child on the swing with someone pushing him to start. The person with the starting push would be the pressure wave, and the cochlear amplification process would be the child pumping to swing higher. This is the vital process that amplifies low intensity sounds, which allows us to hear us wide dynamic

Figure 1.5. Cross section of the cochlear partition. The outer hair cells are colored in dark yellow, and the inner hair cells are colored in orange.
range of sound intensities [1]. As for the inner hair cells, their main function is to transduce the mechanical stimulus at their stereocilia into a signal in the auditory neurons that contact the inner hair cells. The MET process results in the opening of the voltage gated calcium channel that stimulates the nerve to communicate with the brain.

In 1971, Rhode et al showed the compressive nonlinearity at the best frequency with a frequency response measured in live monkeys. This is the frequency-specific cochlear amplification that demonstrated the active MET feedback loop [1]. Because the cochlea is tonotopic, it is of great interest for the auditory research groups to study its different regions. The frequency response at the basal region is different from that of the apical. It is seen at the basal region that there is a high gain at the best frequency for low intensity stimulus. Whereas for the apical region, the active amplification is much smaller, and it is present for all frequencies [1].

To access the sensory tissues in the organ of Corti, it is more straightforward at the basal region through the round window opening, or the apical region through the bone (the bone is relatively thinner there). Anywhere else, the cochlea is surrounded by bones and tissues, opening to access the sensory tissues often makes the cochlea passive. In the thesis, we will talk about imaging and measuring displacement at the sensory tissues with a bulk-optics imaging system (Chapter 3) and with a probe-based system (Chapter 4). Appendices include a chapter that describes an early version of the probe-based system.
CHAPTER 2. *Optical Coherence Tomography system overview*

Understanding the physiology of cochlear mechanics is what our lab is focused on. As an electrical engineer in the lab, I worked on the optical coherence tomography (OCT) system set up. OCT is an imaging modality that is used for cross-sectional imaging. It uses a laser source in the infrared range to penetrate several millimeters into the tissue. The system’s axial resolution is dependent on the central wavelength and the bandwidth of the light source, and the lateral resolution is solely dependent on the optical design, especially the numerical aperture of the objective lens.

### 2.1 Time-domain optical coherence tomography

OCT systems are broadly categorized into two types: Time and Fourier domain systems. For the time-domain OCT (TDOCT) system, first developed in 1991, the reference mirror is scanned mechanically to form the interference pattern when the optical path length (OPL) difference between the mirror and the sample is within the coherence length [3, 4]. The interference signal, termed the fringe, is amplitude modulated with a carrier frequency determined by the scanning speed of the reference arm [3] (Figure 2.1 A). For every scan, the reference mirror needs to move through the entire axial range $d$. The fringe location corresponds to the depth location of the sample reflectors. The three reflectors in the sample (Figure 2.1 B) are shown with the three fringes at the corresponding depth locations, with each fringe intensity dependent predominately on the sample reflectivity and the amount of scattering in the beam path. The fringes are demodulated to form a one-dimensional depth profile, termed the axial scan (A-scan) [2].
Later on, Fercher et al from the University of Vienna found that A-scan can be obtained without having to mechanically scan the reference arm mirror [5]. They noted that the Fourier transform of the interference signal $S(\lambda)$ (with a broadband wavelength range) also generates the A-scan. This finding revolutionized the OCT field, and systems using this technique are called Fourier Domain OCT systems (FDOCT). There are two types of FDOCT systems: Spectral Domain optical coherence tomography (SDOCT) and Swept Source optical coherence Tomography systems (SSOCT). SDOCT system has a broadband source in the wavelength-
domain, and it uses a diffraction grating in the spectrometer to diffract $S(\lambda)$ into its corresponding wavelength pixel [5]. The SSOCT system sweeps across a range of wavenumbers with a tunable laser, and the interference pattern from each wavelength is collected by a detector sequentially (Figure 2.3) [6, 7]. Both systems use a wavelength-multiplexing technique that results in a much higher signal to noise ratio (SNR) than that of TDOCT systems [8, 9]. The Thorlabs Telesto III used in my work is an SDOCT system. This type of OCT is appropriate for our studies because of its superior phase stability compare to that of SSOCT and TDOCT systems [10]. For the SDOCT system, there are no moving parts, and the beam at all wavelengths illuminates the spectrometer simultaneously to create an A-scan.
A

Light of all wavelengths are integrated into a single detector

B

Light is split into M-channels of detectors each corresponding to a different wavelength

C

Light source is split into M-channels of wavelengths
2.3 Physics of how optical coherence tomography operates

Here we describe the basic principle of how the OCT’s sample and reference beams interfere in a simple layered sample, shown in Figure 2.3 [2]. The initial beam from the light source is represented by a plane wave with electric field $E_i(k) = s(k)e^{i(kz-\omega t)}$, with $s(k)$ representing the source spectrum in wavenumber $k$-domain and the exponential terms containing the propagation parameters. After going through the 50/50 beam splitter, the backscattered electric field from the reference mirror is represented as:

$$E_R(k) = \frac{E_i}{\sqrt{2}}r_Re^{-jkz_R}$$

with $r_R$ representing the reference mirror reflectivity, and $z_R$ representing the distance from the beam splitter to the reference mirror.
The backscattered electric field from the sample is:

\[ E_S(k) = \frac{E_i}{\sqrt{2}} [r_s(z_s) \otimes e^{-j2kz_s}] \]

\( \otimes \) is the convolution operator indicating how one function is altered by another function (how the electric field in the sample arm is modified by the sample reflector). \( r_s \) represents the sample reflectivity, and \( z_s \) represents the distance from the beam splitter to the sample.

The combined beam goes to the detector, and creates a photocurrent that is proportional to the square of the electric field sums:

\[ I_D(k) = \frac{\rho}{2} < |E_R + E_S|^2 > = < (E_R + E_S)(E_R + E_S)^* > \]

\( \rho \) is the responsivity of the detector, and the angular bracket indicates the photodetector integration time.
Expanding the equation give three important terms:

\[ I_D(K) = \frac{\rho}{4} [S(k)(R_R + R_{S_1} + R_{S_2} + \cdots)] \quad DC \text{ term} \]

\[ + \frac{\rho}{4} |S(k)| \sum_{n=1}^{N} \sqrt{R_{R_n} R_{S_n}} (e^{i2k(z_{R_n} - z_{S_n})}) \quad cross-correlation \text{ term} \]

\[ + \frac{\rho}{4} |S(k)| \sum_{n \neq m=1}^{N} \sqrt{R_{S_n} R_{S_m}} (e^{i2k(z_{S_n} - z_{S_m})} + e^{-i2k(z_{S_n} - z_{S_m})}) \quad auto-correlation \text{ term} \]

with \( S(K) = < |s(k)|^2 > \)
Since the photodetector is sensitive to the real part of the terms:

\[ I_D(K) = \frac{\rho}{4} [S(k)(R_R + R_{S_1} + R_{S_2} + \cdots)] \quad DC \text{ term} \]

\[ + \frac{\rho}{4} [S(k) \sum_{n=1}^{N} \sqrt{R_{R_n}R_{S_n}} \cos(z_R - z_{S_n})] \quad \text{cross-correlation term} \]

\[ + \frac{\rho}{4} [S(k) \sum_{n \neq m=1}^{N} \sqrt{R_{S_n}R_{S_m}} \cos(z_{S_n} - z_{S_m})] \quad \text{auto-correlation term} \]

The DC term is an offset to the detector current; it is dependent on the sample and reference mirror reflectivity, but not on their OPL difference. The autocorrelation term is the interference between the sample reflectors, and these terms are usually negligible. The cross-correlation term is what forms the interferograms; it encodes the information on the OPL difference between the reference mirror and the sample reflectors [2].
2.4 Optical coherence tomography interferogram and how to interpret it

Let us investigate the interferogram patterns and their physical meaning. When the OPL difference between the sample and the reference arm is small, only a few wavelengths would have the reference and sample arm beams exactly in–phase (when reaching the photodetector) to result in maximum constructive interference. Maximum destructive interference happens when the wavelengths from the reference and sample arms are exactly out-of-phase. There are of course those in-between with varying levels of interference to create a sinusoidal pattern on the spectrometer, show in Figure 2.4. That is why an object closer in depth (smaller OPL difference) has an interferogram lower in spatial frequency (Figure 2.4 left panel); whereas, when the OPL difference is large, more wavelengths would fit exactly in-phase and out-of-phase to form a higher spatial frequency interferogram (Figure 2.4 right panel).

![Figure 2.4](image.png)

Figure 2.4 When the OPL difference is small between the reference and sample arm, only a few wavelengths from the reference and sample arms would be exactly in-phase (when reaching the photodetector) to have maximum constructive interference (left panel). When the wavelengths from the sample and reference arms are exactly out-of-phase, maximum destructive interference occurs. Whereas when the OPL difference is large, there are more wavelengths are in-phase and out-of-phase, to form a higher spatial frequency interferogram (right panel).
An interferogram $I_D(K)$ is the summation of the multiple sinusoids (from reflectors at different depths), shaped by the source spectrum. For simplicity in this schematic we’ve removed the source spectrum shaping. In Figure 2.5 A and B, the spectrometer data of reflector $Z_1$ and $Z_2$ are shown. $Z_1$ is the reflector with the smaller OPL difference since it has the lower spatial frequency. They summed up to form the interferogram in Figure 2.5 C. The Fourier transform of $I_D(K)$ gives the complex A-scan $i_D(z)$, and $|i_D(z)|$ indicates the sample’s depth and reflectivity information.
Figure 2.5 A) The summation of each reflector’s interference pattern (two in this example) to form the interferogram. The inverse Fourier transform of the interferogram gives the complex A-scan $i_D(z)$.

2.5 Parameters of Optical Coherence Tomography system characteristics

When designing the optical Coherence Tomography systems, it is important to determine the system parameters needed for one’s particular application. In this section, axial, lateral resolution, and the depth of focus are discussed.

Figure 2.6 shows the measured source spectrum of our SDOCT system using an optical spectrum analyzer. It has ~15 milli-Watts of power. Shorter wavelength light source has better axial resolution, but it also scatters more, resulting in a smaller penetration depth. This phenomenon is called the Rayleigh scattering. The axial resolution is related to the central wavelength and the $\Delta k$ bandwidth of the source spectrum:

$$\Delta z = \frac{2\sqrt{\ln 2}}{\Delta k} = \frac{2\ln 2 \lambda_0^2}{\pi \Delta \lambda}$$

To have an ultra-high resolution optical system, one could use a super-broadband light with 800 nm wavelength light source [11]. For the lateral resolution, it is decided by the numerical aperture (NA) of the objective lens on the sample arm [2].

$$\delta x = 0.37 \frac{\lambda_0}{NA}$$
Although the axial and lateral resolutions are decoupled in the system design, the axial field of view (FOV) is correlated to the lateral resolution. The axial FOV also determined by the NA of the objective lens, characterized by $FOV_{axial} = \frac{0.5665 \lambda}{\sin^2[\frac{\sin^{-1}(NA)}{2}]}$ [2] For an objective lens with high NA, the system would have a good transverse resolution, but a smaller Rayleigh range (Figure 2.7 B). The Rayleigh range is half of the axial FOV. Whereas an objective lens with low NA, the system would have a large Rayleigh range but a worse transverse resolution (Figure 2.7 A). The tradeoff is seen in Figure 2.7. The objective lens in our bulk-optics system has a NA of 0.062, resulting in an axial FOV of 0.76 mm.
In Chapter 3 and 4, we will discuss the details of how to use the bulk-optics and probe-based systems for imaging and intracochlear measurements. These systems are based on phase sensitive SDOCT, and a literature review of previous applications is included in these chapters.
CHAPTER 3. Adapting a commercial Spectral Domain Optical Coherence Tomography system for time-locked displacement and physiological measurements

The content of this chapter is published in the Mechanics of Hearing conference paper:


The current gold standard for cochlear vibrometry is Laser Doppler Vibrometry (LDV) [12]. We also use a Polytec OFV-5000 in the lab (Figure 3.1). However, the LDV only allows for velocity measurement at one surface at a time and can only access the closest surface of the cochlear partition due to its operating wavelength at 632.8 nm (Helium-neon), which is too short to penetrate tissue significantly.

The introduction of OCT systems is vital to cochlear vibrometry because it has several millimeters of penetration depth to image cochlear structures, and can acquire simultaneous displacement of multiple surfaces within the partition. There are several cochlear mechanics groups that built bench-topped OCT systems for intra-cochlear measurements, starting from the TDOCT systems [13], to SDOCT [14, 15 16], and SSOCT systems [17]. Our purpose of selecting a commercial SDOCT system is so that such valuable cochlear vibrometry/imaging system can be more accessible to auditory researchers who do not have extensive background in optical system building. We detailed the software and hardware steps to modify a commercial SDOCT system for intracochlear displacement in our Mechanics of Hearing conference paper, “Adapting a commercial spectral domain optical coherence tomography system for time-locked displacement and physiological measurements”.

20
The general steps of the SDOCT data processing includes reference beam subtraction, interpolation, and spectral shaping of the spectrometer data to get the complex A-scan. Furthermore, to find the sub-nanometer displacement, we used Spectral Domain Phase Miscoy (SDPM), a function extension of the SDOCT system [18].

We will also describe the hardware and software steps to time-lock the stimulus tones and microphone. As we see in Figure 3.2, the sound-induced fluid traveling wave pushes onto the cochlear structures. Acquiring the simultaneous shearing motions of the Tectorial membrane (TM) and the Reticular Laminar would provide insight on how the cochlear amplification process is
activated. With the current resolution of our system, we can identify motions of different regions of the cochlear partition, as will be discussed later.

![Cross section of the cochlear partition](image)

**Figure 3.2** Cross section of the cochlear partition. The shearing motion of the structures push onto the outer hair cells to activate the mechano-electrical transduction process.

### 3.1 SDOCT real-time B-scan imaging

For real-time B-scan imaging, we use the Thorlabs software *ThorImage*. Its software platform is shown in Figure 3.3. With *ThorImage* we can see the top-down video image of the sample (Figure 3.3) and visualize the B-scan image (cross-sectional image formed by scanning a series of A-scans at different x-positions)
Figure 3.3 The Thorlabs imaging platform, ThorImage, was used to display the B-scan of a gerbil cochlea

For intracochlear experiment, we would first set the B-scan with a lateral FOV of 400 μm for structural recognition. Following that, the FOV is gradually decreased, while keeping the region of interest at the center. The structures of interest have to be at the center because we are acquiring A-scans (FOV = 0) over time, termed M-scans, to observe the sample’s motion. We had our Telesto III sample arm mechanically modified, so the sample beam can be rotated to a desired angle (Figure 3.4).
3.2 SDOCT data processing steps to obtain displacement data

As described above, for SDOCT systems, the sample and reference beams interfere constructively or destructively based on their OPL difference to form the interferogram. The combined beam is sent to a spectrometer, where a diffraction grating separates the beam s(k) into its corresponding individual wavelength on the Linescan camera. The interferogram is first interpolated from the lambda (Figure 3.3 A) to the wavenumber k-domain (Figure 3.3 B), and then inverse Fourier transformed to get the complex A-scan [2]. The magnitude of the complex A-scan indicates the depth profile of the sample (Figure 3.3 C). The phase variation over time at a particular A-scan structure shows its vibration waveform (Figure 3.3 D). The Fourier transform of the vibration waveform show the amplitude, phase, and frequency of the motion (Figure 3.3 E).
3.3 MATLAB data processing steps

The recorded spectrometer data from ThorImage is contained in a zip file, called the .oct file. We will discuss in the next section how to access the photodetector data using C++ platform instead of ThorImage. There are several Matlab processing functions provided by Thorlabs. The flow diagram of the processing steps is shown in Figure 3.6. First, the .oct file is opened with `OCTFileOpen.m`, followed by `OCTFileGetRawData.m` to get the raw photodetector data (2048 lambda-domain photodetectors in the spectrometer) and reference beam (first several A-scans with the galvanometer directed to the side, so only the reference beam is illuminating the spectrometer.). After this, the data is multiplied by the `BinarytoElectronCountScaling` constant (step 1.1 and 1.2

![Figure 3.5](image1.png)
in Figure 3.6). The data is normalized by subtracting the reference beam (step 1.4) and windowed to reduce the system’s point spread function (PSF) side-lobes (step 1.5) [2]. We have attempted several types of windows, and Hanning window was selected because it reduces the side-lobes while keeping the integrity of the data with a flap top [19].

The apodized data in \( \lambda \) is interpolated to the k domain with the chirp vector (step 2 in Figure 3.6). The chirp vector is a numerical transformation (spline function) that converts the data from lambda to k domain while making sure that the interpolated data, in k, is evenly spaced in the k-domain. Each Telesto system has a unique chirp vector that is measured during manufacturing. The interpolated data is then Fourier transformed to get the complex A-scan. The magnitude of the complex A-scan gives the depth profile.
3.4 Use the C++ Software Development Kit (SDK) for Telesto III control

The limitation with using ThorImage is that it can only acquire 10000 maximum continuous A-scans in a M-scan. However, we would like to obtain 1048576 continuous A-scans (approximately 10 seconds of recording) to lower the displacement noise floor. We will discuss how the length of the acquisition time improves the displacement noise floor in the next chapter.
To have a complete data recording of the frequency response of the cochlear structures, we stimulate the ear with multiple frequencies (usually sixty pure-frequency tones) to span the hearing range of the gerbil, and five sound pressure levels at 40, 50, 60, 70, 80 dB. This amounts to three hundred data sets (M-scans) that need to be recorded. We looked into the Thorlabs C++ Software development kit (SDK) in Microsoft Visual Studio to control the software and hardware of the Telesto III. We set up automated looping of data acquisition and created the directory where the OCT data would be exported, with timestamp included for classification. Also, the M-scan is set to have 10 seconds acquisition time, which gives ~0.02 nm displacement noise floor; this is comparable to the displacement noise floor obtained by the vibrometry systems for other research groups [20, 21].

3.5 Time-Locking the stimulus and SDOCT system

The Tucker Davis Technologies (TDT) Real-time Processor RP2 is used to control the speaker and acquire the microphone data. It is important to synchronize the RP2 to the SDOCT system to have time-locked displacement data. The sampling rate is set at 100 kHz (10 μs sampling period), so frequency components up to ~50 kHz can be measured. It was difficult to synchronize the RP2 with the Telesto I, because the Telesto I cannot be externally triggered. The Telesto I uses the NI PCIe-1427 (a Camera link frame grabber) to acquire data from the Goodrich 1024-pixel InGaAs Linescan camera. We tapped the triggering signal from the Linescan camera (in the free-run mode) and input it into the National Instruments cDAQ 9178 to trigger the TDT RP2. However, without knowing the exact starting positioning of the camera’s internal clock, there is always ~15
microseconds uncertainty. We communicated with the Thorlabs engineers with this problem. The Telesto III that came out later has the feature to be externally triggered.

As for the Telesto III, we used the TDT’s RP2 to output a TTL driving signal. The signal is sent through the Camera Link frame grabber PCIe-1433 to trigger the Goodrich 2048-pixel InGaAs Linescan (this is called the asynchronous mode). The finer sampling interval $\delta \lambda_s$ (more pixels in the spectrometer) in $\lambda$ extends the system’s imaging depth $z_{max} = \frac{\lambda_0^2}{4\delta \lambda_s}$ [2], giving our system an imaging range of 3.5 mm. The Linescan camera needs to be triggered by a high duty cycle TTL pulse train because the camera exposure time depends on the length of the “high” signal. RP2’s 100 kHz pulse train is modified with software and hardware circuit to create a high duty-cycle trigger signal. The general triggering set up is shown in Figure 3.7.

![Figure 3.7](image)

*Figure 3.7* The Tucker Davis Technologies Real-time processor is set to have a sampling rate at 100 kHz, (period of 10 us) to control the stimulus tone and microphone. Through software and hardware modification, a high-duty cycle signal is created to trigger the 2048-pixel InGaAs camera.
The TDT RX6 has a sampling period of 10μs, and the goal is to use the TDT’s circuit software RPvdsEx to export a TTL pulse train at twice the sampling period through BitOut. The design circuit schematic, shown in Figure 3.8.

![Design Circuit Schematic](image)

**Figure 3.8** The TDT’s RPvdsEx is used to export the TDT’s 100 kHz clock signal. Sro is used to generate a trigger pulse once the user clicks start. RS and JK flip-flop are used to modify the clock signal, which is exported at BitOut M=1.

Here is the logic behind the design circuit: a one-shot and zBusA are input into a RS flip-flop (zBusA at set, one-shot at reset); the one-shot is used to reset the memory of the RS flip-flop (Q=0) with an impulse. When the rising edge of zBusA is detected (R=0 S=1), a step-function S1 is generated with a rising-edge appearing at the same as that of zBusA (the time diagram of the RS flip-flop is shown in Figure 3.9) [22]. S1 is then input into both J and K of a JK flip-flop to toggle Q (since J=1 K=1) at the sampling rate (timing diagram of the JK flip-flop is in Figure 3.9). The JK flip-flop functions as a toggle controller: moving the pulse up and down every 10 μs [22]. A pulse train T1 with twice the sampling period of RX6 is exported to the PP16 patch port 181.
Since the Telesto III requires a TTL pulse train with a high duty-cycle, T1 needs to be modified for such purpose. The next goal is to create a high duty-cycle pulse train 8 μs high and 2 μs low at the start of rising and falling edges of T1 (Figure 3.10).
I created a customized-built circuit with four XOR gates (CD4077BE chip). T1 is input into three grounded XOR gates to obtain a nano-second time-delay. By inputting T1 and the time-delayed T1 into the fourth XOR gate, a series of impulses at the rising and falling edges of T1 is generated, called T2 (setup shown in Figure 3.11).

**Figure 3.10** The pulse train with a sampling period of 20 μs is sent to a hardware custom rising and falling edge detector and a monostable multi-vibrator to create the high duty cycle pulse train to the Linescan camera.

**Figure 3.11** Custom-made rising and falling edge detector constructed with four XOR gates. The first three XOR gates are used to get a nanoseconds delayed of the input signal T1. By putting T1 and the delayed T1 into the fourth XOR gate, impulses appear at the rising and falling edges of T1.
When inputting T2 into a non-retriggerable monostable multi-vibrator (DM74LS221N) with a resistance value of 150 kΩ and capacitance of 68 pF, a pulse train with an ~8 μs high ~2 μs low is produced to trigger the Linescan Camera, and synchronize it with recordings into the TDT system, such as ear canal pressure.

3.6 Spectral Domain Phase Microscopy for displacement measurement

SDOCT’s functional extension- Spectral Domain Phase Microscopy (SDPM) [23, 24] uses the Doppler-shifted phase signal to determine sample’s sub-nanometer displacement. This vibrometry method is used clinically to look at tissue and cell motions. Some more specific applications will be discussed at the introduction of Chapter 4.

Let us examine the physics of the Doppler-shifted phase signal of one-reflector sample. A reflector located at depth location $Z_0$ would generate a sinusoidal pattern on the spectrometer, described in the interferogram section in Chapter 2:

$$S_{OCT}(k) = A_{OCT}(k) e^{i k [2(Z_0 - Z_{ref})]}$$

$Z_0 - Z_{ref}$ is the OPL difference between the reference and sample arms. Since the axial resolution cannot be infinitely small, $Z_0 - Z_{ref}$ is most likely not exactly one of the $\Delta z$ pixels in the A-scan. With this sub-pixel distance offset $\delta$, an alternative expression $Z_0 - Z_{ref} = Z_p + \delta$ is used, where $Z_p$ is a path length that is an exact multiple of the A-scan $\Delta z$ value(Figure 3.12). Then:

$$S_{OCT}(k) = A_{OCT}(k) e^{i [2kZ_p + [2k\delta]_0]}$$
$k_0$ is the center wavenumber of the light source, and $2kZ_p$ is a phase constant because the only varying variable over time is $\delta$ due to the sample’s sub-pixel motion.

Figure 3.12 The optical path length difference between the sample and reference arms $Z_0 - Z_{\text{ref}}$ is often not exactly on a $\Delta z$ pixel in the A-scan. We can represent it by $Z_0 - Z_{\text{ref}} = Z_p + \delta$, where $Z_p$ is a path length that is an exact multiple of the A-scan pixels. The time variation of $\delta$ would give information on the sample’s motion.

The main purpose of Spectral Domain Phase Microscopy (SDPM) is to find the Doppler-shifted phase change caused by the sample’s motion, so we can call the sample motion as $\delta = \delta_0 + \delta(t)$, and represent the interferogram signal as:

$$S_{\text{OCT}}(k) = A_{\text{OCT}}(k)e^{i[2kZ_p + \Theta(t)]}$$

with $\Theta(t) = 2nk_0\delta_0 + 2nk_0\delta(t) = \theta_0 + 2nk_0\delta(t)$. The time varying term can be determined from the M-scan. The Doppler-shifted phase over time at a particular A-scan structure indicates the structure’s motion. The vibration waveform can be found in the phase variation over time:
\[ \delta(t) = \frac{\theta(t) - \theta_0}{2nk_0} \]

With \( n \) as the refractive index of the medium.

### 3.7 SDOCT system displacement comparison to the LDV

We performed two tests to verify that the Telesto III is time-locked to the TDT system and that the measured displacement is accurate. To do so, the Polytec LDV OFV-5000 and the Telesto III were used to measure the displacement of a Thorlabs piezoelectric actuator. Piezoelectric actuator is composed of ceramic that expands and contracts with stimulated voltages.

In the first measurement, sinusoidal voltages from 5 mV to 4 V at 10 kHz were sent to the piezoelectric actuator. Since its motion corresponds directly to the voltage applied, a linear increasing displacement is expected with increasing voltage magnitude applied. The SDOCT and LDV measured displacements were consistent, as seen in Figure 3.13.
In the second test, fixed amplitude sinusoid voltages with frequencies from 100 Hz to 5 kHz were sent to the piezoelectric actuator. The purpose is to see whether the two systems’ frequency responses are time-locked. After accounting for the instrument delay between the two systems, we found that the LDV phases led the SDOCT phases by a quarter cycle across the frequencies (Figure 3.14). This is expected because the LDV measures velocity and the OCT measures displacement.

![Graph](image)

**Figure 3.13** The LDV and the SDOCT systems were both used to measure the motion of a piezoelectric actuator. The LDV and the Telesto reported almost identical displacements.

**Figure 3.14** The piezoelectric actuator’s frequency response measured by the LDV and the SDOCT systems. The piezoelectric actuator is controlled by fixed amplitude sinusoidal voltages from 100 Hz to 5 kHz. The results show that the LDV leads the SDOCT phases by a quarter cycle. This is expected because the LDV measures velocity, whereas the OCT measures displacement.
3.8 Current research status

My colleague C. Elliott Strimbu is currently using the modified Telesto III system to study various aspects of cochlear mechanics [24, 25]. He set up a broadband 60-frequency tone stimulus Zwuis [26] to cover the frequencies within the gerbil’s hearing range. The frequencies are selected to be unevenly spaced so that there is no overlap of any nonlinear components up to the third order.

Below is an example of his data. First, he acquired a cochlea B-scan for structure recognition. Following that, he reduced the FOV to zero to get the sample beam pointing at the region of interest (Figure 3.15). The BM and outer hair cell regions (asterisks in inset) are then selected to look at their frequency responses stimulated with the Zwuis tone at 5 SPLs (Figure 3.16).

Having this broadband tone is time-efficient. Instead of taking sixty single-tone data sets for 5 amplitudes (300 sets of 10 seconds data), we can take 5 amplitudes of the broadband multi-tone data sets.
Figure 3.15 The B-scan of gerbil cochlea, and the corresponding A-scan at the region of interest after decreasing the FOV. From the A-scan, he selected the BM and the outer hair cell region for their frequency response.
Figure 3.16 The frequency response of the gerbil’s BM and outer hair cell stimulated with the Zwuis tone at 5 sound pressure levels. Note that the motions of the two structures were acquired simultaneously so we could better understand their relative movements.

3.9 Conclusion

In this chapter, we detailed the hardware and software steps to modify a commercial SDOCT system for intracochlear imaging and displacement. Our purpose is to make this powerful system more accessible to auditory scientists, even those without extensive optics background.
CHAPTER 4. Scanning GRIN-probe for in vivo imaging and displacement measurements in the cochlea

The content of this chapter is published in Biomedical Optics Express:


In the Chapter 3, we described how we used the bulk-optics Telesto III SDOCT system to measure the organ of Corti motions through the RWM. This is at the basal region and it does not require drilling into the cochlear bone [19, 24, 25]. Other research groups imaged through the bone at the apical region to measure the organ of Corti motion [17]; such an approach is possible because the bone is relatively thin at that location. So far, these are the two regions accessible for bulk-optics OCT in vivo experiments because the cochlea is surrounded by bones and tissues, and damage to it often make it loses its active amplification process.

Inspired by Dr. Elizabeth Olson’s pressure sensor designed to measure the fluid pressure close to the organ of Corti [27], I decided to make a SDOCT-based displacement fiber-optic sensor. This would give more freedom in accessing the cochlea. This is not the first displacement SDOCT probe, optical coherence elastography uses a fiber optic probe to measure the mechanical tensile and compression characteristics of tissues for detection of specific pathologies, such as tumors and cardiac plaque [28-32]. A piezo-electric probe holder is incorporated to scan two-dimensionally for real-time B-scans. Various kinds of rotating probe are used in the cochlea for circumferential imaging [33, 34], but this probe is the first to both image and measure displacement in the cochlea.

Initially, both ball-lens [35, 36, 37, 38,] and micro Graded index (GRIN) fiber lens [39, 40, 41] probes were designed on Zemax, and sent to WT&T Inc, Canada for fabrication. We decided
on the GRIN lens probe, because of its slim width of 140 μm, which is better for accessing the cochlea.

### 4.1 GRIN-lens probe characteristics

A GRIN lens has refractive indices that decrease with increasing radial distance. When a beam enters the GRIN lens, it travels in a beam path as shown in Figure 4.1. The convergence length is represented by the $g$ factor of the GRIN lens.

![Figure 4.1](image)

**Figure 4.1.** A cartoon showing the beam path in a GRIN lens. Because the GRIN lens refractive indices decrease with increasing radial distance from the optical axis, the beam refocuses with a certain periodicity (determined by the $g$ factor).

The probe is composed of a micro-GRIN lens 140 μm in diameter, with $g = 5.9 \text{ mm}^{-1}$, and 500 μm in length, fused to SMF-28 fiber. The microscope image in the in Figure 4.2 A, and it was used to image a water-immersed mirror to determine the focal length (Figure 4.2 B). The beam profile was measured by the manufacturer with a BeamScan optical profiler at the focal point, and it has a spot size of 11 and 12 μm in the x and y-axes respectively. Since the probe would be used in a fluid environment, we calculated the GRIN lens probe’s theoretical ray tracing matrix analysis,
which will be discussed in the next section [42, 43, 44, 45, 46, 47]. Based on the calculation, the focal length is increased by 1.3 times in water, and the beam waist remains the same.

\[ n'(r) = n(1 - g^2 r^2)^{\frac{1}{2}} \]

**Figure 4.2.** There is a 500 μm long, and 140 μm wide GRIN fiber fused onto a SMF fiber. The probe has a focal length of 250 μm, which matches with the theoretical ray tracing matrix analysis. A. Microscope image of the probe. B. The A-scan of a water-immersed mirror measured by the probe, with a FWHM of 12 μm. C, D. Measured beam profile in the x and y-axes.

To characterized GRIN lens’ gradient index change along the radial axis:
$r$ is the radial position from the middle, $n$ is the refractive index of the middle part of the GRIN lens (1.49 for our GRIN lens), $z_w$ (found in calculation below) is the final beam waist location (where the sample is), and $g$ is the GRIN lens parameter ($5.9 \text{ mm}^{-1}$ for our GRIN lens).

### 4.2 Ray Tracing Matrix GRIN lens

The GRIN lens probe is sectioned into several interfaces for the theoretical ray tracing matrix calculation, as shown in Figure 4.3. The representation of each matrix in shown below:

![Figure 4.3](image)

**Figure 4.3.** Schematic for the ray-tracing matrix of a GRIN probe. First the light goes through the SMF, no-core fiber (NCF), GRIN fiber, and sample mirror. Since we are interested in the beam after reflecting from a sample, we use a perfectly perpendicular flat mirror as the sample.
The total beam path can be represented by the $M_{56}M_{45}M_{34}M_{23}M_{12}$. With the output $q_2$ and input $q_1$ included:

$$q_2 = \frac{Aq_1 + B}{Cq_1 + D}$$
\[ A = \cos(gl) - \frac{n}{n_2}z_w g \sin(gl) \]

\[ B = \left( l_0 + \frac{n_1}{n_2}z_w \right) \cos(gl) + \left( \frac{n_1}{n_g} - \frac{ngl_0 z_w}{n_2} \right) \sin(gl) \]

\[ C = -\frac{ng}{n_2} \sin(gl) \]

\[ D = -\frac{ngl_0}{n_2} \sin(gl) + \frac{n_1}{n_2} \cos(gl) \]

Plugging the ABCD values give us \( q_2 \), and that is used to determine \( w_{01} \):

\[ w_{01} = w_0 \left( \frac{n_1}{n_2} A^2 + \frac{a^2 B^2}{AD - BC} \right)^{\frac{1}{2}} \]

With \( a \) being:

\[ a = \frac{\lambda}{\pi w_0^2 n_1} = \frac{a_0}{n_1} \]

Since our targeted focal length is \(~250 \, \mu m\), we do not need to use the NCF for beam expansion.

We can set \( l_0 = 0 \):

\[ z_w(l_0 = 0) = \frac{n_2 \left[ 1 - \left( \frac{a_0}{ng} \right)^2 \right] \sin(gl) \cos(gl)}{ng[\sin^2(gl) + \left( \frac{a_0}{ng} \right)^2 \cos^2(gl)]} \]

And the corresponding waist size is:

\[ w_{01}(l_0 = 0) = \frac{a_0 w_0}{ng[\sin^2(gl) + \left( \frac{a_0}{ng} \right)^2 \cos^2(gl)]^{\frac{1}{2}}} \]
Where \( w_0 \) is the initial beam width entering the GRIN lens.

Using these formulas, the beam waist is expected to be \(~6.5\ \mu m\) and the focal length \(~112\ \mu m\). The manufacturer-measured beam waist size \(11\ \mu m\) and the focal length \(250\ \mu m\) are slightly different. According to the manufacturer, the discrepancy comes from the fusing process of the SMF and the GRIN lens: there is a mixed region combining the SMF and GRIN lens, instead of a clear boundary used in the calculation. Due to this technicality, the theoretical calculation helps with determining the approximate dimensions of the GRIN lens probe, but the fabrication process has to be partially dependent on trial-and-error to obtain the desired beam characteristics.

4.3 Piezoelectric probe scanner setup

In order to use the probe for imaging, we developed a one-dimensional scanning probe holder to have a controllable lateral scanning range up to \(380\ \mu m\). The probe holder is made of a piezoelectric bimorph with dimensions of \(2.5\ cm \times 0.6\ cm \times 0.05\ cm\), with a resonant frequency of \(~270\ Hz\), calculated from the defined PZT-5H material constants [48]. A piezoelectric bimorph is a material that bends laterally with the voltage applied, and it is attached to an aluminum rod that is held by a three-dimensional micromanipulator (purchased at World Precision Instruments) for maneuvering. The probe tip in this design bends \(~2\ \mu m\) per volt. The photograph of the probe setup is shown in Figure 4.4 A), and B) shows the probe insertion location during in vivo experiments.
In order to use the ThorImage real-time B-scans software platform, the driving signal for the Telesto III x-axis galvanometer is tapped. The driving signal is modified and sent to the piezoelectric bender for scanning. The following section describes how the trigger waveform for the x-axis galvanometer is extracted, processed, and used to control the scanning probe. The flow diagram of the modification circuitry is shown in Figure 4.5.

For imaging with the probe, the sampling rate is set to 10 kHz, which results in a 1 second scan rate. The tapped driving signal is a 1 second periodic sawtooth waveform with a spike at the
end of each sawtooth (Figure 4.5 A) The spike is used to divert the galvanometer away from the sample to acquire the reference beam, and can be turned off in the Thorlabs setting configuration. However, we did not turn it off in software, because we switch between the bulk-optics system and probe often. The spike contains high frequency components that resulted in problematic ringing of the piezoelectric bimorph, so the goal outlined in Fig. 4.5 is not only to eliminate the spike but also to low-pass filter the waveform to decrease the high frequency components. The waveform modification steps are labelled sequentially in Roman numerals in the paragraphs below, with some waveform illustrations simulated with LTspice circuit software for demonstration, as the National Instruments DAQ cannot take in signals over ±10 volts.

Figure 4.5. Flow diagram of the circuitry modification steps to change the tapped galvanometer driving signal to control the scanning of the piezoelectric probe holder
4.3.I The original sawtooth driving waveform tapped using a tee-shaped SMB from the Telesto III x-galvanometer is shown in Figure 4.6. The large spike at the end of each sawtooth waveform is used to divert the sample arm so only the reference beam is recorded onto the spectrometer. There are many frequency components at the ~270 Hz resonance frequency of the piezoelectric bender.

![Figure 4.6. A) One period of the NI DAQ-recorded sawtooth with spike waveform](image)

4.3.II A summing amplifier was used to amplify X10 the original sawtooth waveform (also invert it), and DC shift to saturate out the spike (Figure 4.7). The following theoretical waveforms generated by LTspice. The summing amp, circuit diagram drawn in the LTspice software (Figure 4.8).
Figure 4.7. We used a summing amplifier to saturate out the spikes from the sawtooth waveform.

Figure 4.8. Circuit diagram of the summing amplifier
4.3.III A low-pass RC filter was used to decrease the ringing of the piezoelectric bimorph, caused by the high frequency components (sharp edges) in the sawtooth waveform (red in Figure 4.9). The low-pass filtered signal is shown in blue. The designed low pass filter cut-off frequency is

\[ f_{c1} = \frac{1}{2\pi R_1 C_1} = 2.84 \text{ Hz}, \text{ with } R_1 = 56 \text{ k}\Omega, \ C_1 = 1\mu F. \]

![Figure 4.9](image-url)

**Figure 4.9.** A low-pass RC filter was used to smoothen the sharp edges of the sawtooth waveform (red). The filtered waveform is shown in blue.

4.3.IV An AC coupler was used to center the sawtooth waveform at zero. The AC coupler filters out frequency below \( f_{c2} = \frac{1}{2\pi R_2 C_2} = 0.0053 \text{ Hz}, \text{ with } C_2 = 1\mu F, \ R_2 = 30 \text{ M}\Omega. \) There is a gradual DC shift in the waveform (over time) because of the capacitor’s transient.
An operational amplifier is used as a voltage follower for buffering (Figure 4.11). The NI DAQ-recorded modified sawtooth waveform is shown in Figure 4.12 in the time and frequency domain. The circuit schematic showing the usage of low pass filter, AC coupler, and voltage follower is shown in Figure 4.13.

Figure 4.10. An AC coupler was used to remove the DC component, centering the waveform at zero (red). There is a gradual DC shift initially because of the capacitor’s transient

4.3. V

Figure 4.11. Operational amplifier as voltage follower
Figure 4.12. The NI DAQ-recorded modified waveform is shown in both the time and frequency domain. The frequency components near the piezoelectric bender’s resonant frequency are low.

Figure 4.13. The circuit schematic of the components to modify the sawtooth waveform from the Telesto x-scanner.
A variable voltage-divider was made (with a potentiometer) to change the amplitude of the sawtooth wave, which controls the lateral scanning range of the piezoelectric bender. The modified sawtooth waveform (the output in Figure 4.12) has voltage amplitude of ±9. The signal is voltage-divided with a 10-turn 2500 \( \Omega \) potentiometer and a 6800 \( \Omega \) resistor so we can control the sawtooth waveform amplitude (Figure 4.14). The minimum amplitude of ±0.13 volts is achieved with this setup. The calculation is shown below [49]:

\[
V_{\text{sawtooth}} = V_{\text{sawtooth max}} \left( \frac{R_p}{R_1 + R_p} \right) = 9 \left( \frac{100}{6800 + 100} \right) = 0.13 \text{ volt}
\]

![Circuit Schematic](image)

**Figure. 4.14** The circuit schematic for the variable voltage divider. It is made with a resistor and a potentiometer, and it outputs the sawtooth waveform with amplitudes ranging from ±0.13 to ±9 volts.
The circuit that starts from the x-galvanometer driving signal, filter and coupling, to acquiring the varying sawtooth amplitude waveform is shown in Figure 4.15.

![Circuit Diagram](image)

**Figure 4.15** The circuit setup to acquire the varying amplitude sawtooth waveform.

4.3.VII To produce the desired DC voltage to control the angle of the piezoelectric bender, we used a variable voltage divider tapping the ±35 volts from the power supply. It uses a 10-turn 2500 Ω potentiometer and two 3900 Ω resistors. The circuit was designed to obtain ±8 V in order to apply a static angle to the piezoelectric bender. The circuit schematic is shown in
Figure 4.16. The resistance of the potentiometer $R_P = R_A + R_B$ is separated into $R_A + R_B$ in the calculation:

$$V_{DC,\text{max}} = \left(\frac{R_2 + R_B}{(R_2 + R_B) + (R_1 + R_A)}\right) 70 \ \text{(volts)} - 35(\text{volts}) \left(\frac{3900 + 2500}{(3900 + 2500) + (3900 + 100)}\right) 70 - 35 = 8 \ \text{volts}$$

$$V_{DC,\text{min}} = \left(\frac{3900 + 100}{(3900 + 2500) + (3900 + 100)}\right) 70 \ \text{(volts)} - 35(\text{volts}) = -8 \ \text{volts}$$

This means that the DC voltage can go from -8 to 8 volts, by changing the $R_B$ value (turning the knob of the potentiometer).

![Diagram](image_url)

**Figure. 4.16.** The circuit schematic of creating the DC voltage $V_{DC}$. The resistance of the potentiometer $R_P = R_A + R_B$ is separated into $R_A + R_B$ since a ±35 volts power source is used. We can generate any DC voltage between ±8 volts to control the piezoelectric bender.
4.3 VII To combine the DC voltage (to control the probe angle) and the varying-amplitude sawtooth waveform (to control the probe’s lateral scanning range), we used a summing amplifier. The circuit diagram of the whole setup is shown in Figure 5.17. The signal output is sent to a x20 piezo driver (Piezo System s, EPA-008-I), which controls the piezoelectric probe holder scanning range.

Figure. 4.17. The circuit setup that includes creating a DC voltage (to control the probe’s angle) and sawtooth waveform (to control the probe’s lateral scanning range) from the galvanometer driving signal.
4.4 Probe imaging and displacement optical setup

The optical setup for imaging is with the noncommon-path (nonCP) configuration using an external reference arm consisting of a polarization controller, collimator, iris diaphragm, and a retro-reflector (Figure 4.18). Having an external reference arm allow us to control the reference beam intensity by changing the angle of the retro reflector or altering the size of the iris diaphragm. This is especially important for optimizing the real time B-scan.

Figure 4.18. Non-commonpath optical setup for imaging uses an external reference arm containing a polarization controller, collimator, iris diaphragm, and a retro-reflector. This configuration allows for fine-tuning of the reference beam intensity through changing the size of the iris diaphragm or the angle of the retro-reflector.

The displacement data are acquired at 97 kHz sampling rate in order to measure displacement frequency up to ~50 kHz. For displacement measurement, we use the common-path (CP) configuration (Figure 4.19 A) to maximize the amount of light to the sample. In the common-path configuration the reference beam is the reflection from the probe/fluid interface. This is an
important step because the camera integration time is significantly shorter during displacement measurement. CP setup uses the partial back reflection from the probe’s front surface as the reference beam (Figure 4.19B). One major advantage of the CP setup is that dispersion compensation is not needed between the reference and sample arms.

**Figure 4.19.** Commonpath optical setup for displacement measurement at 100 kHz. Fiber-probe set-up. For comparison, the Telesto’s standard free-space operation is indicated in the left-bottom gray-dashed-box inset. Then the Telesto base unit is connected via a single mode optic fiber (blue dashed line) to the Thorlabs’ probe head, which has an internal reference arm (green dotted). For the common-path fiber probe operation (dashed box in the middle of the figure), one attaches the fiber optic probe to the Telesto base unit. The probe has a self-contained reference arm from the partial back reflection from its front surface. This reference arm is indicated in the green-dashed line. The sample path is indicated in the red solid line. In either case, for synchronizing the Telesto to the TDT stimulus and acquisition system, the pulse train from the TDT RP2 is modified in hardware and software circuitry to drive the Telesto III base unit.
The data processing between the bulk-optics and probe SDOCT systems is the same, except for one step. It is how one acquires the background signal. For the Telesto III SDOCT system, the galvanometer is diverted to the side to acquire the reference beam alone before each B-scan. For the probe system, the probe is placed in a medium similar to that of the sample fluid (water) and far from any reflecting surfaces before the experiment to acquire the background signal.

4.5 Dispersion Compensation for imaging

With the external reference arm configuration we used for imaging, dispersion mismatch between the two arms needs to be accounted for since it causes broadening of the features in the A-scan, and thus diminishes axial resolution. Dispersion results from the wavelength dependence in the speed of light through different materials [51-57]. A dispersion correction can be applied after data acquisition, which we briefly review here. The phase \( \theta(k) \) of the \( S(k) \) signal, obtained with the Hilbert transform of \( S(k) \), can be represented by a Taylor series expansion. In the absence of dispersion, \( \theta(k) \) of a specular reflector is linear in \( k \). The presence of terms second order and above is due to dispersion [50, 51]. Dispersion can be reduced by doing a polynomial fit of the signal from a specular reflector, and then compensating terms in the \( \theta(k) \) function that are second order and above [50, 51]. To illustrate, we acquired an A-scan of a specular reflector with the bulk-optics SDOCT system (yellow in Figure 4.20) and added a sine-shaped dispersion \( \theta_{\text{dispersion}}(k) \) term to the \( S(k) \). The resulting A-scan from the dispersed data had a broadened structural peak (Figure 4.20 red). We corrected the \( \theta_{\text{dispersion}}(k) \) and were able to recover an A-scan that overlays with the original (blue), with a slightly higher noise floor. This experiment was to demonstrate the broadening effect of dispersion mismatch.
Another option for dispersion compensation is using the *ThorImage*’s built-in dispersion compensation software, which uses a specular structure from the sample to determine the second order correction term in $\theta(k)$. The surface of the piezoelectric actuator and the round window membrane (RWM) in the gerbil’s cochlea served as the well-reflecting structures for our purpose. With *ThorImage*, we were able to look at the A-scan of the sample in real time while applying different values of the second order term in $\theta(k)$. The second order term that resulted in the sharpest A-scan peak from the well-reflecting structure was exported and used to dispersion-compensate our data. The correction calculation, in terms of the Telesto III line-camera pixel

![Figure 4.20](image-url)
number \( n \), is

\[
\theta(n) = a_2 \left[ \frac{n-N}{N} \right]^2,
\]

where \( a_2 \) is the second order term, \( N \) is the total number of photodetectors (2048 for our Line Scan camera), and \( n \) is the \( n^{th} \) pixel on the line-camera. This phase correction was applied to the complex \( S(k) \) data by multiplying by \( e^{-i\theta(n)} \). Finally, taking the real part gives the dispersion compensated \( S(k) \) \cite{50, 51}. Dispersion compensation was applied to B-scan acquired from the nonCP setup. The example below in Figure 4.21 demonstrates the effect of dispersion compensation. The A-scan was taken with the probe to look at a water immersed reflective sample (Figure 4.21 blue). By applying the correct dispersion factor, a sharper structure is recovered (Figure 4.21 red), the magnified A-scan is shown in Figure 4.21 B).

![Figure 4.21. An A-scan of a water-immersed specular reflector taken by the GRIN-lens probe (blue) with the nonCP setup. By applying a second order dispersion correcting phase term, we achieved a sharper and higher amplitude peak (red).](image)
4.6 Probe demonstration with two in vivo experiments

The functionality of the probe is demonstrated with two in vivo experiments done on Mongolian gerbils. To stimulate the ear, a speaker tube is coupled to a Fostex tweeter, with the microphone placed inside the ear canal. Zwuis, the broadband tone described in Chapter 3, was used to stimulate the ear at various SPLs.

4.6.1 Probe displacement noise floor experiment

In the first experiment, the probe was inserted through the round window to image through the RWM, shown with the gray dotted box in the cross-sectional cochlea sketch (Figure 4.22 A). The probe was initially scanning at ~380 μm to acquire the B-scan (Figure 4.22 B). The magnified cochlea sketch (Figure 4.22 A) was used to identify the cochlear structures. From the top down we saw: RWM, BM, organ of Corti, and Reissner’s membrane.

Once that is done, the lateral scanning range was gradually reduced (by decreasing the amplitude of the sawtooth waveform) while angling the probe to center at the structures of interest (with the DC voltage). The probe ultimately stopped at the desired location (shown with the red dotted line in Figure 4.22 B) for displacement measurement. The inset in Figure 4.22 B shows the corresponding A-scan of the red dotted line, with the RWM at 300 μm, organ of Corti complex structures between 400-500 μm, and the Reissner’s membrane at 650 μm.
Figure 4.22 A. A sketch of the cochlea cross-section. The probe is inserted through the most basal region of the cochlea, shown in the gray dotted box. The boxed region is magnified for structural recognition. B) The B-scan image obtained with the scanning probe, and it shows similar structures as in Figure 5.21 A). The lateral field of view is gradually decreased and ultimately stopped at the red-dotted line for displacement measurement. The corresponding A-scan from the red-dotted line is shown in the inset.
In Figure 4.23 A, the microphone-recorded ~ 60 dB SPL Zwuis stimulus tone frequency spectrum is shown. The BM is selected for the analysis (black dot in the Figure 4.22 B A-scan). The BM’s Fourier-transformed vibration waveform, termed the frequency response, is shown in Figure 4.23 B. It has a displacement noise floor of ~0.02 nm, comparable to other SDOCT systems [16, 17, 19]. A peak at 25 kHz is presented in the frequency response. This result is comparable with measurements made by other groups at the same cochlear region [58, 59, 60]. The goal of the experiment is to demonstrate the probe’s functionality, and new physiology is not the goal.

![Figure 4.23 A. The Fourier-transformed stimulus broadband Zwuis tone at 60 dB SPL. B. The Fourier-transformed vibration response of the BM. It shows a passive response with a peak at 25 kHz. This result is comparable to those made by other research groups. The displacement noise floor is ~0.02 nm.](image)
4.6. II Displacement comparison between the bulk-optics and the probe systems

For the second in vivo experiment, we verified the displacement consistency of the bulk-optics SDOCT and probe systems. Both systems were used to measure displacement inside the organ of Corti complex. We started with the bulk-optics SDOCT system to image for structural recognition (Figure 4.24), followed by displacement measurements with the Zwuis tone stimulus. The probe was then inserted at the same location and angle to acquire displacement measurements. The relative beam position of the bulk-optics SDOCT system is shown in the blue dotted line, and that of the probe is shown in red, based on their respective A-scans (presented on the sides of the B-scan). The A-scans are positioned vertically to match with the structures in the B-scan.

The most reflective point in the organ of Corti is selected for both A-scans (the color dots) for the measured frequency responses. In the frequency response plot in Figure 4.24 B, the gain is referenced to the stapes motion for normalization, and the phase is referenced to the ear canal pressure. The overall results are similar for both systems, and the slight difference in the amount of gain is likely due to the small (~ 7 μm) lateral positional difference.
Figure 4.24 A The B-scan taken with the bulk-optics SDOCT system. The bulk-optics and probe SDOCT systems are used for displacement comparison. The relative beam position of the bulk optics system (blue) and the probe (red) are shown, based on the measured A-scans on the left and right of the B-scan. B) The displacement frequency response of the most reflective structure is compared, and their magnitudes and phase are similar.
4.7. Discussion

To determine the phase noise of an optical coherence tomography system, one could measure the phase variations of the top and bottom surfaces of a coverslip over time [61]. Ideally, the phase difference should be constant over time since it is based on the OPL between the coverslip surfaces, but it fluctuates due to the system’s phase noise. To estimate the signal’s phase noise, the standard deviation of the phase difference over time was found to calculate the minimum detectable phase difference, or phase sensitivity, $\sigma_{\Delta \phi}$. The A-scan SNR was found by dividing the intensity at the peak of interest by the standard deviation of the noise. The theoretical prediction of the phase sensitivity was discussed by Choma [61], making

$$
\sigma_{\Delta \phi} = \frac{2}{\pi} \left[ \frac{1}{SNR} \right]^{\frac{1}{2}}.
$$

This gives the displacement sensitivity in the time domain, $\delta x_{sens \_time} = \frac{i}{4n \pi} \left[ \sigma_{\Delta \phi} \right] = \frac{i}{4n \pi} \left( \frac{2}{\pi} \right) \left[ \frac{1}{SNR} \right]^{\frac{1}{2}} [61, 62, 63]$. The A-scan SNR correlates directly to the displacement noise floor $\delta x$ (for phase-sensitive SDOCT displacement measurement): a higher A-scan SNR would result in a lower displacement noise floor.

The predicted $\delta x_{sens \_time}$ for the cochlear structure in Figure 4.22 is $\frac{1300 \ nm}{4 \times 1.3 \times \pi} \left( \frac{2}{\pi} \right) \left( \frac{1}{52.5} \right)^{\frac{1}{2}} \approx 7 \ nm$, using the equation above. The measured vibration waveform was Fourier transformed to distribute the noise among all the frequency bins (524288); thus, lowering the noise floor by a factor of $\frac{1}{\sqrt{\text{number of frequency bins}}}$. Data acquisition time lowers the displacement noise floor because there are more frequency bins present. The theoretical $\delta x_{sens \_time}$ comes out to be 0.01 nm, which is the same as the experimental value in Figure 4.23 B.

It is difficult to get a high SNR (low displacement noise floor) deep inside the organ of Corti because most of the sample beam is scattered off by that point. Additionally, the sensory tissues
have low reflectivity. For example, the outer hair cell reflectivity is only 0.006% [64]. At the apical region of the cochlea, Recio-Spinoso and Oghalai observed displacements of tens to hundreds of nm at low to moderate SPLs [65], so the displacement noise floor in that region is less of a concern. As for the basal region, the cochlear structures move at sub-nanometer scale with low SPLs. In order to maximize the A-scan SNR (to get a lower displacement noise floor), we switched from nonCP to CP setup to avoid losing the sample beam going to-and-forth from the 75:25 fiber coupler. The 0.02 nm displacement noise floor was obtained with the CP setup (Figure 4.22 B). With this, we can typically measure frequency response from stimulus tones down to 40 dB SPL.

4.8. Conclusion and future direction

In this chapter, we described a SDOCT-based probe for intra-cochlear imaging and displacement measurement. For the next step, we would like to attach an electrode to the probe. Doing so, we could to measure spatially and temporally coherent displacement and voltage data (from the outer hair cells) to further understand the active electro-mechanical transduction (MET) feedback loop. A similar dual sensor, containing the electrode and pressure sensor, has been used in our lab. [66].

4.9. Impact

The scanning probe podium talk was enthusiastically received at the 2019 Association for Research in Otolaryngology conference. Many professors came to discuss potential collaboration opportunities. I am currently assisting my colleague Elika to perform physiology study with the probe.
The Appendices include a chapter that details an earlier design of a water-immersed SDOCT probe.

CHAPTER 5 Signal Competition in the Heterodyne interferometer and the SDOCT system

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Both the heterodyne interferometers and SDOCT systems measure displacement using the same fundamental principle: the Doppler-shifted phase in the signal. Since we gave a detailed description on the basic principles of how SDOCT systems operate in Chapter 2, I am going to give an overview of how heterodyne interferometers operate for comparison.

5.1 Basic principle of how a heterodyne interferometer measures velocity

A heterodyne interferometry system has been the gold standard for cochlear vibrometry for decades [67, 68, 69]. It uses a He-Ne laser with wavelength of 633 nm. The beam is initially separated into sample and reference beams, with a frequency offset in between beams: \( \omega_s \) and \( \omega_r \). After the sample beam reflects off the sample, and reference beam off the mirror, the two beams recombine and strike the photodetector. The important part of the recombined signal has a carrier frequency of \( \omega_c = \omega_o - \omega_r \), and the signal is represented as:

\[
S_{HI}(t) = A_{HI} e^{i(\omega_c t + \phi_{oo})}
\]

where \( \phi_{oo} \) is a phase constant. \( A_{HI} \) is the signal intensity, which depends on the power of the light source and the sample reflectivity.
When the sample beam reflects off of the moving sample, there is a Doppler frequency shift of:

$$\Delta \omega(t) = 2v(t) \frac{\omega_0}{c}$$

where \(v(t)\) is the velocity at which the sample moves, and the factor of two accounts for the to-and-fro of the light beam path. When the expression above is plugged in, the signal looks like:

$$S_{HI}(t) = A_{HI}e^{\left\{i\left(\omega c t + \frac{2\omega_0}{c} \int v(t')dt' + \phi_0 \right)\right\}}.$$ 

which equals to:

$$S_{HI}(t) = A_{HI}e^{\left\{i\left(\omega c t + \phi(t)\right)\right\}}$$

with \(\phi(t) = \frac{2\omega_0}{c} \int_0^t v(t')dt' + \phi_0\).

Taking the time integral gives \(\phi(t) = \frac{2\omega_0}{c} \delta(t) + \phi_0\), where \(\delta(t)\) is the object’s displacement and \(\phi_0\) is a constant. Using the relationship \(\frac{\omega_0}{c} = k_0\):

$$\phi(t) = 2k_0 \delta(t) + \phi_0$$

$$S_{HI}(t) = A_{HI}e^{\left\{i\left(\omega c t + 2k_0 \delta(t) + \phi_0\right)\right\}}.$$ 

The displacement is encoded in the phase \(\phi(t)\):

$$\delta(t) = \frac{\phi(t) - \phi_0}{2k_0}$$

Thus, the displacement is encoded in the phase, just as in the SDOCT system.
5.2 Signal Competition

There have been multiple published papers showing the SDOCT-measured intra-cochlear frequency responses. In some papers, the reported displacement from every A-scan pixel is plotted. Our lab decided to investigate the validity of such approach since phase-leakage (also termed signal competition), between neighboring A-scan pixels, has been reported by Ellerbee et al [70]. They stated that the signal from a reflector would influence the reported phase in the neighboring pixels, and this was observed when they studied the motion of cardiomyocytes under a glass coverslip. The bright reflection from the coverslip influenced the reported displacement of the cells 40 μm away. Our lab had recognized a similar phenomenon with the heterodyne interferometer: signal from an out-of-focus reflector was influencing the signal in focus [69].

These results -- where motion from one location influences the reported motion in a nearby location, appear similar in nature. In this chapter, we will demonstrate the similarity between how the SDOCT systems and heterodyne interferometers obtain displacement.

5.3 Signal competition in LDV system two-reflector theoretical calculations:

When operating the heterodyne interferometer, the sample beam is focused on the structure of interest (object A), and the Doppler shifted phase is encoded in the signal. However, when there are multiple reflectors neighboring each other, the situation becomes more complicated. The returned signal would contain the signals from both objects, represented by:

\[
A_{HI}e^{i(\omega_c t + \phi_A(t))} + B_{HI}e^{i(\omega_c t + \phi_B(t))} = e^{i\omega_c t} (A_{HI}e^{i\phi_A(t)} + B_{HI}e^{i\phi_B(t)})
\]

Having N reflectors mean that we would sum the N terms (instead of the two terms in this case) into the equation above. We are going to look into this two-reflector case for demonstration.
We can factor out the common carrier frequency term $e^{i\omega_c t}$, and represent the signal with real and imaginary terms based on the Euler’s formula:

$$A_{HI}e^{(i\phi_A(t))} = A_{HI} \cos(\phi_A(t)) + iA_{HI} \sin(\phi_A(t))$$

$$B_{HI}e^{(i\phi_B(t))} = B_{HI} \cos(\phi_B(t)) + iB_{HI} \sin(\phi_B(t))$$

So the combined signal $A_{HI}e^{(i\phi_A(t))} + B_{HI}e^{(i\phi_B(t))}$ is:

$$= A_{HI} \cos(\phi_A(t)) + B_{HI} \cos(\phi_B(t)) + i[A_{HI} \sin(\phi_A(t)) + B_{HI} \sin(\phi_B(t))]$$

From this, the phase is:

$$\phi_T(t) = \arctan\left\{\frac{A_{HI} \sin(\phi_A(t)) + B_{HI} \sin(\phi_B(t))}{A_{HI} \cos(\phi_A(t)) + B_{HI} \cos(\phi_B(t))}\right\}$$

The amplitude of $A_{HI}$ and $B_{HI}$ depends on how reflective the objects are and the optical sectioning capability of the system.

If there were only one single reflector, then $\phi_{A0}$ would not have any influence on the reported displacement; however, with multiple reflectors, the phases of the reflectors, $\phi_{A0}$ and $\phi_{B0}$ are important.

Let’s evaluate the equation without the $\phi_{A0}$ and $\phi_{B0}$, and use small angle approximation to gauge the reported phase:

$$\phi_T(t) \approx \frac{A_{HI}\phi_A(t) + B_{HI} \phi_B(t)}{A_{HI} + B_{HI}}$$
The reported phase in this approximation is a weighted sum of the phase from object A and B. It is an intuitive way to think about how signal competition work. However, $\emptyset_{A0}$ and $\emptyset_{B0}$ are extremely influential in the reported value of $\emptyset_T(t)$, and in most cases it is not appropriate to use a small angle approximation.

**5.4 Signal competition in SDPM:**

Let’s consider the two-reflector case like we did for the heterodyne system, and we would like to find the displacement at location $Z_0$ (in between object A and B). The signals from object A and B contribute to the reported signal. Again, the amounts of signals from the objects depend on the sample structure reflectivity and the PSF of the light source. The width of the PSF is determined by the optical bandwidth: the wider bandwidth correlates to narrower PSF. The FWHM of our system is $\sim 6 \mu m$.

![Figure 5.1. The Telesto III system’s point-spread-function](image.png)

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\( A_{OCT, Z_0} \) and \( B_{OCT, Z_0} \) at \( Z_0 \) pixel is represented by:

\[
A_{OCT, Z_0} e^{i[2k(Z_0 - Z_{\text{ref}}) + \Theta_A]} + B_{OCT, Z_0} e^{i[2k(Z_0 - Z_{\text{ref}}) + \Theta_B]}
\]

\[
= e^{i[2k(Z_0 - Z_{\text{ref}})]} [A_{OCT, Z_0} e^{i\Theta_A} + B_{OCT, Z_0} e^{i\Theta_B}]
\]

The exponential term \( e^{i[2k(Z_0 - Z_{\text{ref}})]} \) indicates the depth location of the \( Z_0 \) pixel. \( A_{OCT, Z_0} \) and \( B_{OCT, Z_0} \) are the objects’ signal strength at the \( Z_0 \) pixel. Their amplitudes are determined by their corresponding A-scan object’s intensity, and how much the signal strength have fallen-off based on their objects’ distance from \( Z_0 \) (found from the PSF in Figure 5.1). For example, an object A with A-scan intensity of 100 would only have a signal strength \( A_{OCT, Z_0} \) of 0.5 if \( Z_0 \) is 10 \( \mu \)m away.

Even if the optical sectioning curve is steep, an object with strong reflectivity would still influence signals from its neighboring pixels. For example, if the signal from object B is around 30 percent the amplitude based on the PSF and three times as reflective as object A, the two objects would roughly have the same amount of influence to the signal at \( Z_0 \) (Figure 5.2),
The other terms are separated into real and imaginary terms based on the Euler’s formula, and the combined phase signal $\Theta_T(t)$ at $Z_0$ pixel is represented by:

$$
\Theta_T(t) = \arctan\left\{\frac{A_{OCT,Z_0} \sin(\Theta_A) + B_{OCT,Z_0} \sin(\Theta_B)}{A_{OCT,Z_0} \cos(\Theta_A) + B_{OCT,Z_0} \cos(\Theta_B)}\right\}
$$

With $\Theta_A = 2k_0\delta_A(t) + \Theta_{A0}$ and $\Theta_B = 2k_0\delta_B(t) + \Theta_{B0}$.

The equation looks extremely similar to that of heterodyne interferometer two-reflector case; thus, the signal competition phenomenon would be similar for both types of systems.
To better visualize how the signals from object A and B combine, we can see them in a phasor diagram, as shown in Figure 5.3. The magnitude of the object A (green) and B’s (red) phasor at $Z_0$ pixel are $A_{OCT,Z_0}$ and $B_{OCT,Z_0}$, and their phase in the exponential term dictate the angle at which the phasor point to. The combined signal C, has a magnitude less than either object, and with a different phase. The phase of C is what are interested in, and it is based on the magnitude and phase of object A and B. This phasor diagram applies to the LDV signals as well.

![Figure 5.3.](image)

**Figure 5.3.** The phasor diagram of adding the signals from object A and B. $A_{OCT,Z_0}$ and $B_{OCT,Z_0}$ represent the magnitude of the phasors, and their phase signals dictate the angle at which the phasors point toward. The combined signal, C, has a magnitude less than that of A and B and with a different phase. The phase of C is what we are interested in, and it is dependent on the magnitude and phase of object A and B.
5.5 Demonstration of signal competition with a two-reflector test sample

To demonstrate the SDOCT signal competition phenomenon, a two-reflector test sample was made. The sample is a glass-tube 2426 μm diameter wide, with a transparent polymer membrane on top and a bare fiber 60 μm behind (shown with the cartoon in Figure 5.4). The fabrication of the sample starts with ultra-violet curing of a 1-μm thick optical adhesive (Norland Products, Cranbury, NJ) in a beaker of water. A glass tube is then plunged into the water (with adhesive film on top) to form the membrane on its tip. We then threaded a bare fiber from the back with a micromanipulator (Marzhauser, Germany).

![Diagram](image)

**Figure 5.4.** The test sample is made of glass tube, with a polymer membrane at the tip and a bare fiber 60 microns behind. We use it to demonstrate phase leakage by analyzing the reported displacement at and between the fiber and the membrane.
The A-scan image of the test sample is shown in Figure 5.5 A). There we see two bright reflectors, the membrane (on the left) and the bare fiber (on the right), separated by a distance of \( \sim 55 \, \mu\text{m} \). In Figure 5.5 B), the microscope image of the sample is shown.

![A-scan image and microscope image](image)

**Figure 5.5.** A) The A-scan of the test sample, with the bare fiber as the first reflector and the membrane as the second reflector. B) The microscope image of the test sample.

The sample is then simulated with pure tones, generated by a signal generator (Wavetek) and audio amplifier (TDT HB7), in an open-field speaker setup (Fostex FT17H). We are interested in knowing what the reported displacement values are at and between the reflectors. Based on de La Rochefoucauld et al, there is a distinction between the signal competition phenomena based on how much the sample moves: when the sample motion is more than \( \frac{\lambda}{10} \) (130 nm for our SDOCT), then it is considered a high modulation index case. When the sample moves less than 130 nm, it is categorized as low modulation index. More detail will be discussed in the next section.
5.5 I High Modulation index case

The A-scan in Figure 5.5 A shows the two reflectors, with the fiber at pixel 367, and the membrane at pixel 377. The distance between them is 11 pixels. The reported vibration waveforms at and between the two-reflectors, indicated with locations 1-4 in the A-scan are shown in Figure 5.6 B, C, D, E)
Figure 5.6. A) The A-scan of the test sample, with the first reflector as the bare fiber and the second reflector as the membrane. It was stimulated with a 7008 Hz pure tone to show the reported vibration waveforms for the high modulation index case. The reported vibration waveforms at depth locations 1, 2, 3, and 4 are shown in B, C, D, E). Note the significant harmonics produced at points 2 and 3. The harmonics are not produced by the speaker since the reported membrane vibration waveform in E) is a perfect sinusoid at 7008 Hz. The green is the theoretical prediction based on the equation, and the free parameter is the phase offset $\Delta \Theta_{AB}$. $\Delta \Theta_{AB} = 1.35$ radian gives the most similar displacement pattern F) We show the frequency response of the reported vibration waveform at point 3, and we not only see the fundamental frequency, but also the harmonics.

Figure 5.6 E) shows the reported vibration waveform of the membrane (red). Figure 5.6 B) shows the reported vibration waveform of the fiber.

What is interesting is the reported displacement at and between the two reflectors. The space in-between is supposed to be air, and it should not have any reported vibration waveform; however, the reported vibration waveform at point 2 contains harmonics (Figure 5.6 C). This is not harmonics created by the speaker because a 7008 Hz pure sinusoid is reported at the membrane in Figure 5.6 E). The harmonics components are even more prominent in the reported vibration waveform at point 3 (Figure 5.6 D).

In Figure 5.6 F), the frequency response of point 3 is shown. There the fundamental frequency at 7008 Hz, in addition to the second, third, fourth and fifth harmonics components are prominent. The amplitude and phase of the fundamental frequency and its harmonics are extracted to create a cleaner vibration waveform for visualization in red, without the influence of the noise.

We then used the prediction equation to generate the theoretical vibration waveform in green. $A_{OCT,Z_0}$ and $B_{OCT,Z_0}$ are estimated based on the reflector’s A-scan intensity and the system’s optical sectioning curve. The free parameter for the simulation is the phase $\Theta_A$ and $\Theta_B$, and the
equation can be simplified so that only their difference $\Delta \theta_{AB}$ is the free parameter. All possible values for $\Delta \theta_{AB}$ were tried out to see which would create a vibration waveform that most resembles the experimental data. A theoretical vibration waveform similar to the experimental data was obtained when the phase difference $\Delta \theta_{AB} = 1.35$ radian.

5.5 II Low Modulation index case

We also demonstrated the low modulation index case with the same test sample stimulated at a lower sound pressure level. The membrane’s motion was 19 nm, and the reported vibration waveforms in-between the reflectors were not distorted. In Figure 5.7 A), the A-scan of the test sample indicates that the fiber is at index 255 and the membrane is at index 265. The reported displacement at and in-between the reflectors are plotted in red in Figure 5.7 B. At indices 256-258, the reported displacements are similar to that of the fiber. At indices 262-264, the reported displacements are similar to that of the membrane. The interesting displacement data is at pixel 259, there is a high amplitude, larger than either motion of the reflectors.

The green is the theoretical value generated with the signal competition equation, using the free parameter $\Delta \theta_{AB}$. The theoretical displacements at and between the reflectors were most similar to the experimental data when $\Delta \theta_{AB} = 2.74$. The conclusion learned from the test sample is that the interesting harmonics for the high modulation case, or the abnormal amplitudes for the low modulation case could simply be caused by signal competition. This is especially important for cochlear vibrometry research: the troughs next to a bright structure may report “interesting results”, which can be interpreted as "interesting cochlear processing” that could be an artifact caused by the more reflective neighbor.
Figure 5.7. A) The A-scan of the test sample, showing the bare fiber and the membrane. The low modulation index case is demonstrated in this experiment. B) By using different $\Delta \Theta_{AB}$ to plug into the equation, we could get different amount of reported displacements at and in-between the reflectors. $\Delta \Theta_{AB} = 2.74$ gives the closest approximation to the experimental data, where there is a large reported displacement at pixel 259.
5.6 Demonstration of signal competition on a cochlea

To analyze how signal competition influence SDOCT intracochlear measurements, we did a signal competition demonstration on an ex vivo gerbil cochlea. We accessed the cochlea at the most basal region imaging through the RWM, as seen in the cochlea cross-section schematic in Figure 5.8 A). In Figure 5.8 B), a B-scan was taken with the bulk-optics SDOCT system, and the FOV is set to zero to acquire M-scan at the red dotted line. The A-scan is shown in Figure 5.8 C), and we separated it into two regions: BM and OCC regions, boxed off in gray. They are viewed as two distinct two-reflector cases for this demonstration. We assumed that the two prominent structures are the only reflectors present.

In Figure 5.8 D), the BM region has two reflectors at depth index 125 and 128 (shown with black dots). The reported displacement (red) shows similar trend as that of the test sample for the low modulation index case, with the neighboring pixels reporting similar displacement as that of the closest reflector. There is a reported displacement at pixel 126 larger than either motion of the reflectors. One could propose that the cochlear structures are demonstrating interesting motion, but most likely it is caused by the signal from the neighboring reflectors.

As for the OCC region, the reported displacements at and between reflector 3 and 4 are shown in Figure 5.8 E). Different from the BM region, the reported displacement at pixel 142 reports a small displacement value. We could model such displacement pattern with the theoretical model, with $\Delta \theta_{AB} = 3$ radian (purple). Note that an even better approximation can be generated when the relative contributions of the two reflectors are changed by a small factor (less than two), shown in green.
Figure 5.8. A. Sketch of the cochlear cross-section B. B-scan of the ex vivo gerbil, with round window membrane, BM, OCC, and Reissner’s membrane surfaces in view. The location of the A-scan is indicated with the red line C. The A-scan is separated into BM and OCC regions. These regions are used as distinct two-reflector examples D, E. The reported experimental displacements are shown in red, and the theoretical models with different phase offsets are plotted in different colors for comparison.
5.7 Discussion

The similarity of the phase-based signal competition is seen between the heterodyne interferometer and the SDOCT-based SDPM through how the signals from the reflectors are summed.

The study is extremely important because many groups were analyzing the reported vibration waveform/displacement from all the depth pixels for their intracochlear measurements. Learning about signal competition gives an idea of how some of the “interesting” results could be erroneous. We concluded that it is “safe” to select the local maximum in the A-scan, since signal competition would not be an issue unless a major reflector is neighboring it. To represent our signal competition findings graphically, a look-up-chart was made (Figure 5.9). The object of interest is A, and object B is the contaminating neighboring reflector. One needs to determine one’s system PSF to calculate the \( A_{OCT,Z_0} \) and \( B_{OCT,Z_0} \). For illustration, we have displacement ratio of 5 (object B moves 5 times as much as object A), and displacement ratio of 0.05 (object A moves more than object B). The y-axis shows the signal ratio \( B_{OCT,Z_0}/A_{OCT,Z_0} \) from small to large. The three sections of the charts show the reported theoretical displacements when three different phase offsets \( \Delta \Theta_{AB} \) are used as the parameter.

To understand what the plot means in dB scale, 0 dB at the fundamental frequency means that the reported and theoretical displacements are the same. If it is 4 dB at the fundamental frequency, it means that the reported displacement is 4 dB greater than the actual displacement. The -40 dB 2\textsuperscript{nd} harmonics curve indicates that the reported displacement at the 2\textsuperscript{nd} harmonics is 40 dB less than the actual displacements at the fundamental frequency.

Not only is this a useful look-up chart to estimate the amount of signal competition possible in the measurement, but we also learned that as long as the signal ratio is below 0.1 and motion is
less than 100 nm, the signal competition effect is negligible. This can be seen in the first and second row in Figure 5.9, the displacements of the harmonic components are at least 25 dB less than that of the fundamental frequency when the signal ratio is below 0.1.

Does signal competition affect the reported phase on a sample that spans several depth pixels? No, it would not. When $\delta_A(t) = \delta_B(t)$, that exponential term separates from the influence of $\Delta \Theta_{AB}$. The signal would be:

$$e^{i(2k(Z_0-Z_{ref}))}e^{i(2k\delta_A(t))}[A_{OCT,Z_0}e^{i\Theta_A} + B_{OCT,Z_0}e^{i\Theta_B}]$$

Figure 5.9. A lookup chart for signal competition equation with a few different possible parameters. The top panel shows the actual displacement of object A and B, with different $\Delta \Theta_{AB}$ as the parameter. The x-axis is the ratio of the signal from object B (contaminator) to object A. The y-axis shows the difference between the reported displacement from the actual displacement, in addition to showing signal competition-created harmonics components.
The reported motion is in the time-varying second term, so the phase offset does not influence the reported displacement.

5.8 Conclusion

Knowing the system’s PSF and sample’s structures reflectivity, one can deconvolve the signal competition prediction equation to figure what the actual motion is at every pixel in the A-scan. The computing steps for this is however time-consuming. Our current approach is to select the local maxima as the structures of interest and make sure no bright reflectors are around to influence the reported displacement.

5.9 Impact

The conclusion we have reached to select the local maxima in the A-scan is widely accepted in the SDOCT cochlear mechanics community. Our paper has been cited ten times in the past three years [71-80].
CHAPTER 6 Conclusion

The three projects I did were customizing a commercial optical coherence tomography system for cochlear vibrometry measurement, fabricating an intra-cochlear scanning SDOCT probe, and analyzing signal competition experimentally and theoretically.

I customized a commercial Spectral Domain Optical Coherence Tomography system for cochlear vibrometry measurements, and detailed the hardware and software steps in our conference paper so auditory researcher, even those without extensive optics background can have access to such a powerful system. Currently, my colleague C. Elliot Strimbu is doing physiology studies with the system.

Still, the cochlea is surrounded by bones and tissues, and the bulk-optics SDOCT systems are limited to accessing the sensory tissues either at the basal or apical regions. That was why I made a scanning SDOCT-probe to access more regions of the cochlea through drilling a hole. The probe would scan to acquire B-scan for structural recognition, followed by displacement measurements. What is exciting about the probe is that electrode can be attached to acquire simultaneous voltage and displacement data. This would provide insights to the mechano-electrical transduction process. My colleague Elika Fallah has started doing physiology studies with this probe-based system.

Lastly, I investigated how the phase-sensitive displacement data is influenced by its neighboring signals. Ellerbee et al first noted the signal competition issue where the SDOCT phase signal from a bright reflector influenced the reported motions of the neighboring cells. I modelled the signal competition phenomenon and compared the result to the experimental data. It was
decided that as long as the local maxima in the A-scan are selected, signal competition is not an issue. Currently, I am collaborating with Dr. Yuye Ling to perform demodulation of the reported phase to recover the actual sample’s phase.


[64]. Acta Oto-laryngologica Supplement 467: Cellular Vibration and Motility in the Organ of Corti (Lund, 1989)


Appendices

I will describe the development and testing of a lens-less displacement probe. The coupling efficiency of this probe was not high due to the lack of a focusing element, and that is why we moved to developing the GRIN-lens probe described in Chapter 4.

A.1 Displacement sensor setup

The sensor, made of single mode fiber (SMF) 125 μm in diameter with 9 μm core, uses a common-path SDOCT configuration (Figure 4.2 A). With this setup, no matching of the sample and reference arms’ OPL is needed, nor does one need to perform dispersion compensation. The common-path SMF probe uses the partial back-reflection from the tip of the probe (fiber/environment interface) as the reference beam, which can be determined using Fresnel’s equation: \( R = \left| \frac{n_1 - n_2}{n_1 + n_2} \right|^2 \). When a common-path probe is used in an air medium, the reflectance at the probe/air interface is ~3.5%, which provides adequate reference beam. However, the sensor is used in the intra-cochlear fluid, only ~0.2% of the incident light would be back-reflected. That is not enough reference beam.

To increase the partial back reflection, Han et al [79] evaporated a thin gold layer at the tip of the SMF probe, and used it to image the frogs’ retina under water. The thickness of the gold evaporated determines the amount of back reflection. Based on the thin-film reflectance calculation [80], we evaporated ~4 angstrom of gold to have a reflection of ~3% of the incident light in water.
A.2 Sensor’s coupling efficiency

To increase the stability and ease of holding of the probe, a pulled glass capillary was adhered 1 cm behind the probe tip. The sample beam fans out because there is no focusing component (Figure A.1 B.). We are going to discuss the coupling efficiency of the SMF probe in the next section.

\[ E(x, y, z) = E_0(z)e^{\frac{ik(x^2+y^2)}{2q(z)}} \]

$q$ is the Gaussian beam complex parameter represented by:

\[ \frac{1}{q(z)} = \frac{1}{R(z)} - j\frac{\lambda}{\pi w^2(z)} \]

Figure A.1. A) Micrograph of the SDOCT probe B) Without a focusing component, the sample beam expands as it leaves the SMF
With $R(z)$ being the beam radius of the curvature and $w(z)$ of the beam width.

\[
\begin{bmatrix}
1 & d \\
0 & 1
\end{bmatrix}
\quad \text{Propagation through free space after exiting the SMF}
\]

\[
\begin{bmatrix}
1 & 0 \\
0 & 1
\end{bmatrix}
\quad \text{Flat mirror reflection}
\]

\[
\begin{bmatrix}
1 & d \\
0 & 1
\end{bmatrix}
\quad \text{Propagation through free space after reflected from the mirror}
\]

\[
M_{\text{Final}} = M_{34}M_{23}M_{12} =
\begin{bmatrix}
1 & 2d \\
0 & 1
\end{bmatrix}
\]

The output Gaussian beam $q_2$ is represented by:

\[
q_2 = \frac{Aq_1 + B}{Cq_1 + D}
\]

Combining the two equations would give us the final beam width $w_2$ at $q_2$:

\[
w_2^2 = \frac{B^2 + A^2 \frac{\pi^2}{\lambda^2} w_0^4}{(AD - BC)(\frac{\pi^2}{\lambda^2} w_0^2)}
\]
After plugging in the ABCD variables:

\[
w_2^2 = 4d^2 + \frac{\pi^2}{\lambda^2} w_0^4 - \frac{\pi^2}{\lambda^2} w_0^2
\]

The Rayleigh range is the distance from the beam waist where the beam radius is increased by a factor of \(\sqrt{2}\). It is \(z_0 = \frac{\pi^2}{\lambda^2} w_0^2\) for this SMF probe (Figure A.2).

**Figure A.2.** Gaussian beam with the beam waist \(w_0\). The Rayleigh range \(Z_R\) is the axial distance at which \(w_0\) is increased by a factor of \(\sqrt{2}\).

With the SMF’s small aperture ~0.14 and the lack of a focusing component, the coupling efficiency gets lower as the sample gets further away.
A.3 Displacement Verification

We verified the reported displacement made by the sensor (used in both air and water), the LDV, and the bulk-optics SDOCT by measuring the motion of a piezoelectric actuator. Note that three of the systems were used in air, while the displacement sensor was used in water. The refractive index $n$ of different mediums is accounted for with the SDPM phase-to-displacement conversion factor $\frac{\lambda_0}{4\pi n}$.

The shaker was driven at 1 volt from 5 to 25 kHz at 1 kHz interval, the frequency response of each system is shown in Figure A.3. The displacement frequency responses match up well. As for the phase of the motion (referenced to the driving voltage to the piezoelectric actuator), the LDV-measured phase led that of the SDOCT-based systems by a quarter cycle. This was seen in Chapter 3 when the LDV was compared to the bulk-optics system.
The displacement sensor with gold on its tip was immersed in water to measure the displacement of the piezoelectric actuator. The measured frequency response is compared to that measured with the bulk-optics SDOCT system, displacement sensor without gold, and LDV (all performed in air). The piezoelectric actuator was driven at 1 volt from 5 to 25 kHz, and the magnitude of the frequency response measured by all modalities match up well. The LDV-measured motion phase led the phase measured with the SDOCT-based systems by a quarter cycle. This is expected because the LDV measures velocity whereas the SDPM measures displacement.

**Figure A.3.** The displacement sensor with gold on its tip was immersed in water to measure the displacement of the piezoelectric actuator. The measured frequency response is compared to that measured with the bulk-optics SDOCT system, displacement sensor without gold, and LDV (all performed in air). The piezoelectric actuator was driven at 1 volt from 5 to 25 kHz, and the magnitude of the frequency response measured by all modalities match up well. The LDV-measured motion phase led the phase measured with the SDOCT-based systems by a quarter cycle. This is expected because the LDV measures velocity whereas the SDPM measures displacement.
A.4 Phase noise

To determine the phase noise of this displacement sensor, I measured the phase variations of the top and bottom surfaces of a coverslip over time (Figure A.4 B). Ideally, the phase difference should be constant over time since it is based on the OPL between the coverslip surfaces, but it fluctuates due to the system’s phase noise. Experimentally, we looked at a thin coverslip using a SDOCT probe, and moved the coverslip to various axial distances to obtain different signal-to-noise ratio (SNR) levels. The samples’ A-scan is shown in Figure A.4 A and C. The coverslip in Figure A.4 A has higher SNR because it is closer to the sample and thus much more reflected light returns to the fiber (Figure A.1B). To estimate the signal’s phase noise, the standard deviation of the phase difference over time (Figure A.4 B and D) was found by calculating the minimum detectable phase difference $\sigma_{\Delta \phi}$. $\sigma_{\Delta \phi}$ is plotted versus the A-scan’s SNR in Figure A.4 E. The experimental $\sigma_{\Delta \phi}$ (circle in Figure A.4 E) matches up reasonably well with the theoretical $\sigma_{\Delta \phi}$ calculated from the equation in Section 4.6: $\sigma_{\Delta \phi} = \frac{2}{\pi} \left[ \frac{1}{\text{SNR}} \right]^{1/2}$ (line in Figure A.4 E).
Figure A.4. A) The coverslip’s A-scan measured with the SDOCT probe. The cover slip and the SDOCT probe were placed close together to get a relatively high SNR B) With the placement as in (A), the phase variation of the top and bottom surfaces over time was plotted along with the phase difference. The standard deviation of the phase difference gives the experimental minimum detectable phase $\sigma_{\Delta \phi}$. C) The SNR was reduced compared to (A) by increasing the distance between the probe and the coverslip. D) The experimental phase variation and difference with the placement as in (C). E) $x_{\text{sens.time}}$ calculated from the $\sigma_{\Delta \phi}$ (circles), matches up reasonably well with the theoretical estimation (line). The data in A and B are represented at the second data point from the right, and the data in C and D are represented at the fourth data point from the left.
A.5 In vivo experiment

The in vivo cochlear measurements were made on a Mongolian gerbil (Meriones unguiculatus). The experimental protocol was approved by the Institutional Animal Care and Use Committee of Columbia University. The animal was anesthetized throughout the experiment, and euthanized at the end. The gerbil’s head was attached to an angle-varying stage with dental cement for easier manipulation. A tracheotomy was performed to provide the gerbil with a clear airway, and the right pinna was also removed. A RadioShack speaker delivered sound stimuli through a tube in the ear canal. A hole (~150 μm in diameter) in the cochlear bone was drilled at the first turn location for insertion of the pressure sensor and then the SDOCT probe.

To confirm the SMF probe-measured displacement, the pressure sensor was used to first determine the BM displacement from the pressure gradient measured close to the BM, using the differential fluid pressures measured at several locations close to the Basilar membrane to calculate the BM velocity. The goal is to place the pressure sensor and the SMF probe at the same location and angle and determine the velocity (with the pressure sensor) and displacement (with the displacement sensor) for frequency response comparison. We stimulated pure tones (1 to 20 kHz at 1 kHz interval) at 60, 80, and 90 dB SPL in the ear canal of the gerbil.

We observed a tuned response when using the pressure sensor, with peak frequency of 12 kHz at 60 dB and 11 kHz at 80 dB (squares in Figure. A.5 D). In the region of the peak and slightly above it, the responses showed compressive nonlinearity. From ~ 5 -9 kHz the responses were linear. At frequencies below that, the pressure difference was small and the displacement found with pressure differences was not reliable. The sizes of the response are similar to measurements in the literature. The phase accumulation through several cycles seen in Figure A.5E is the signature of the cochlear traveling wave.
After the pressure sensor was removed, the SDOCT probe was inserted from the same hole (1 second data acquisition times). The probe was advanced to a position ~ 20 μm from the first structure in the A-scan, the putative BM. At 60 and 80 dB SPLs, the vibration signals were not out of the noise. The measured BM frequency response at 90 dB SPL (black circles in Figure A.5D) is tuned with a peak at 10 kHz, and shows similar traveling wave delay to the displacement measured with the pressure sensor. The motion of a location ~ 35 μm behind the BM was similar in size and also showed traveling wave delay. These observations speak to the basic utility of the displacement sensor. However, the sensor-measured displacements were smaller than what had been measured with the pressure-gradient method, and smaller than what was expected from the values in the literature. There is no technical reason for the lower displacements and it is probable that the probe was not centered on the BM, but was closer to the ~ fixed lateral boundary.
A.6 Discussion and Conclusion

In this work, we demonstrated a common-path SMF probe for phase-sensitive displacement measurements in a liquid medium. The common-path probe setup is straightforward, without any need to match the reference and sample arm lengths or correct for polarization difference between the two arms.

Without a focusing element though, it is difficult to measure displacement deep inside the organ of Corti. That is why we developed the GRIN-lens probe discussed in Chapter 4.

Figure A.5.A) The measured A-scan of an in vivo gerbil cochlea, with the BM region of the (circle) the first structure, and the deeper-OC region (triangle) behind it. B) The frequency spectrum of the BM at 9 kHz. The noise floor is ~ 0.09 nm C) The frequency spectrum of the deeper location at 9 kHz. The noise floor is ~ 0.17 nm. D) The pressure sensor-measured BM displacement at 60 and 80 dB SPLs (squares) compared with the SDOCT probe measured BM (circle) and deeper-OC (triangle) displacements at 90 dB SPL. E) All measurements of the structures have the phase accumulation that is characteristic of the cochlear traveling wave.
Peer review journal article and conferences

Peer-reviewed journal articles


Conference paper


Conference presentation


Nathan C. Lin, C. P. Hendon, E. S. Olson, “Using a commercially available Optical Coherence Tomography system to measure vibrations within the Cochlea,” Association for Research in Otolaryngology (ARO), Poster presentation (2016).