

**APPLICATION OF MULTI-TONAL COMPLEX WITH OPTICAL  
COHERENCE TOMOGRAPHY IMAGING SYSTEM FOR  
VIBROMETRIC ANALYSIS OF INNER-EAR STRUCTURES**

An Undergraduate Research Scholars Thesis

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## **ABSTRACT**

Application of Multi-Tonal Complex with Optical Coherence Tomography Imaging System for Vibrometric Analysis of Inner-Ear Structures

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Currently, research is being conducted on single tone sound transduction within the auditory portion of the inner ear, the cochlea [1]. The study of cochlear mechanics in large rodents has provided important insight into cochlear function [1]. Additionally, multi-tone stimuli can provide a new perspective in vibrometric analyses that could be used in audiometry as a measure of hearing loss for patients in terms of both magnitude and their unique frequency envelope. Therefore, the proposed study was aimed to test the recent implementation of multitone calibration into an existing python program, PyVib2, which was used during Optical Coherence Tomography (OCT) imaging experiments for vibrometric analysis of the structures in the inner-ear. Within PyVib2, the multi-tone stimulus program will be executed in conjunction with the OCT M-scan program for full vibrometric analysis. This research resulted in the further development of the PyVib2 program, the acquisition of insightful image data of structures of the human inner-ear, which was collected using the OCT imaging system, and the verification of extracting the vibratory response of these structures when subjected to a multi-tone stimuli.

## **ACKNOWLEDGEMENTS**

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# CHAPTER I

## INTRODUCTION

Currently, research is being conducted on sound transduction within the auditory portion of the inner ear, the cochlea [1]. The study of cochlear mechanics in large rodents has provided important insight into cochlear function [1]. The study of cochlear mechanics is made possible with the use of spectral domain optical coherence tomography to visualize and measure sound-induced vibrations of intracochlear tissues (shown in Figure 1) [1].

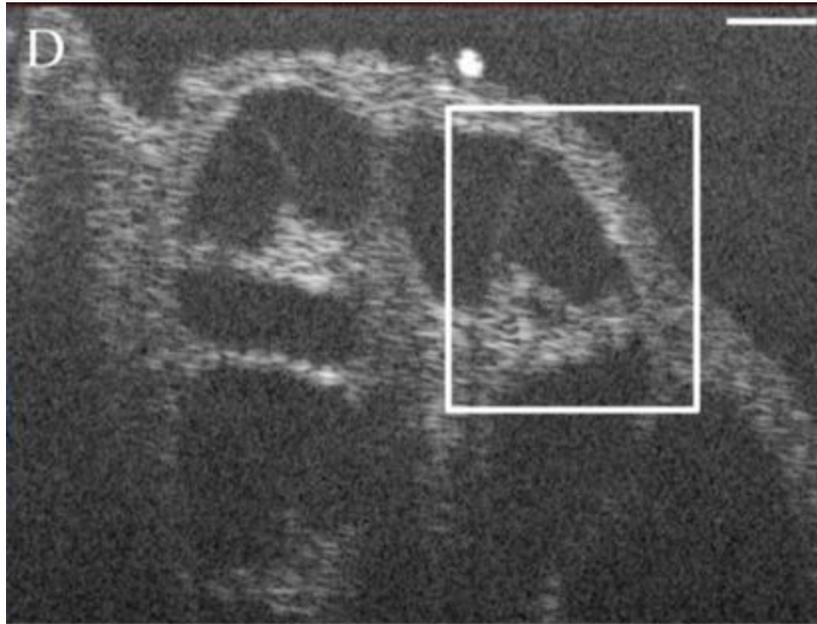


Figure 1. OCT cross-sectional B-scan of a fixed and decalcified cochlea in vitro [1]. The structures of interest are boxed [1]. The scale bar is 200  $\mu\text{m}$  [1]. Structures of interest are the Reissner's membrane, the organ of Corti, basilar membrane, and the auditory nerve fiber.

The sounds used to induce vibrations are pure tone stimuli, which are sinusoidal waveforms of any frequency, phase, and amplitude. Additionally, multi-tone stimuli can provide a new perspective in vibrometric analyses. The definition of a multi-tone stimulus is a combination of two or more pure tone stimuli; therefore, this stimulus contains more than one frequency component. The first part of this proposed study was aimed to implement multitone calibration into an existing python program, PyVib2, which was used during OCT imaging experiments for vibrometric analysis of the cochlea.

Vibrometric analyses have potential for use in the audiometry room. The current standard for measuring the magnitude of hearing loss in a patient is the pure tone test, which involves playing an individual tone and prompting the patient to answer “yes” or “no” to whether they heard the sound or not. This method does not provide a quantifiable metric of hearing loss and requires at least 30 minutes for a thorough investigation of the patient’s frequency envelope. Therefore, the second part of this proposed study was aimed for the verification that the vibrometric data extracted from the raw data collected from a handheld OCT device could reveal the frequency response of inner-ear structures such as the tympanic membrane, incus and the stapes [2]. This method would allow a patient's hearing loss to be accurately quantified in matter of seconds with the magnitude of the patient’s hearing loss as well as the patient’s specific frequency envelope, which could also be used to assist the audiologist in the fine tuning of hearing aids for the patient.

## CHAPTER II

### METHODS

In order to carry out the first part of this research project, the fundamentals of tone signals and multi-tone signals were investigated and understood. The definition of a multi-tone signal is a combination of two or more pure tone stimuli. A multi-tone signal contains more than one frequency component. Furthermore, the “Signal Sampling Theorem” states that a continuous time signal can be represented in its samples and can be recovered back when sampling frequency  $f_s$  is greater than or equal to twice the highest frequency component of message signal. Therefore, this theorem theoretically explains how a multi-tone signal is sampled in a digital environment to provide a digital representation of the desired signal. Equations 1, 2, and 3 provide the mathematical explanation for the signal sampling theorem. Equation 1 represents the original multi-tone signal,  $x(t)$ .

$$x(t) = a_1 \cos(2\pi f_1 t) + a_2 \cos(2\pi f_2 t) + a_3 \cos(2\pi f_3 t) \quad (1)$$

Equation 2 represents the Fourier transform of the multi-tone signal.

$$X(f) = \frac{a_1}{2} [\delta(f - f_1) + \delta(f + f_1)] + \frac{a_2}{2} [\delta(f - f_2) + \delta(f + f_2)] + \frac{a_3}{2} [\delta(f - f_3) + \delta(f + f_3)] \quad (2)$$

Equation 3 represents the Fourier transform of the multi-tone signal when sampled at a given sampling rate  $f_s$ .

$$X(f) = \frac{1}{T_s} \left( \frac{a_1}{2} [\delta(f - f_1 - f_s n) + \delta(f + f_1 - f_s n)] + \frac{a_2}{2} [\delta(f - f_2 - f_s n) + \delta(f + f_2 - f_s n)] \right. \\ \left. + \frac{a_3}{2} [\delta(f - f_3 - f_s n) + \delta(f + f_3 - f_s n)] \right) \quad (3)$$

Based on the assumption that  $a_1 < a_2 < a_3$ ,  $f_1 < f_2 < f_3$ , and  $fs > 2f_3$ ,  $X_s(f)$  was plotted manually (shown in Figure 2).

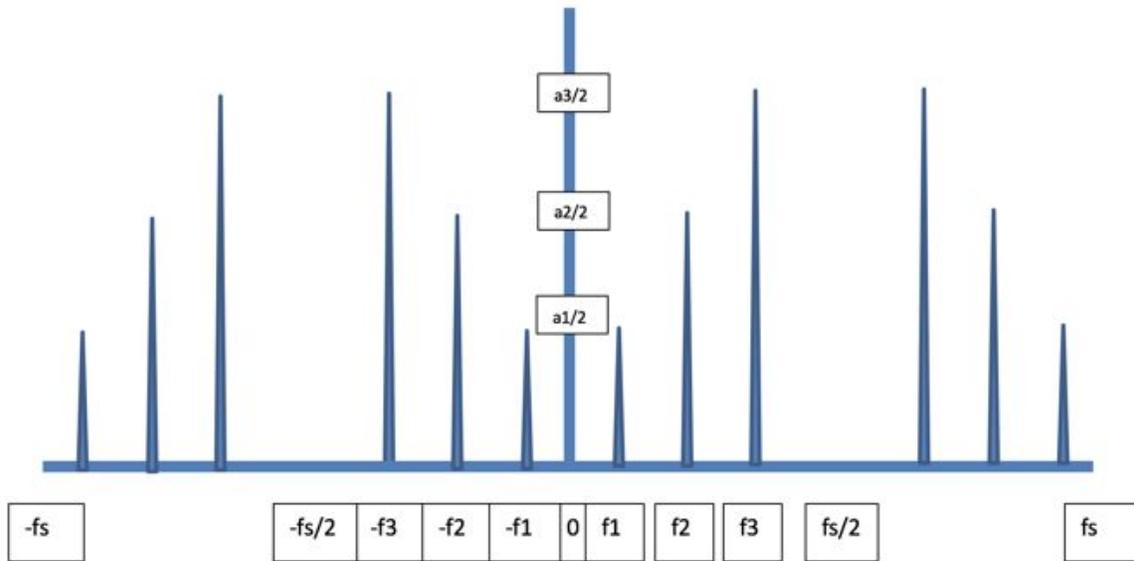
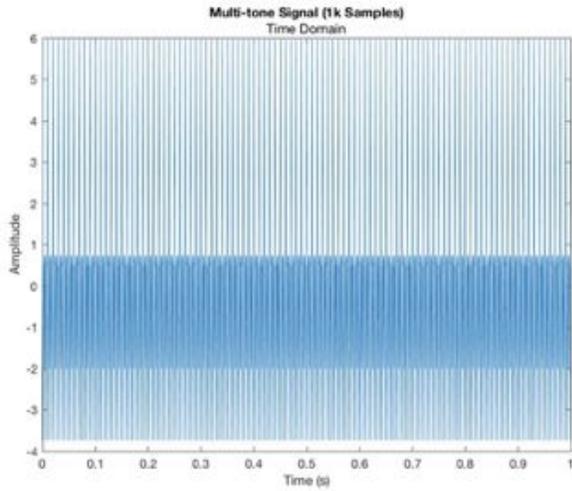
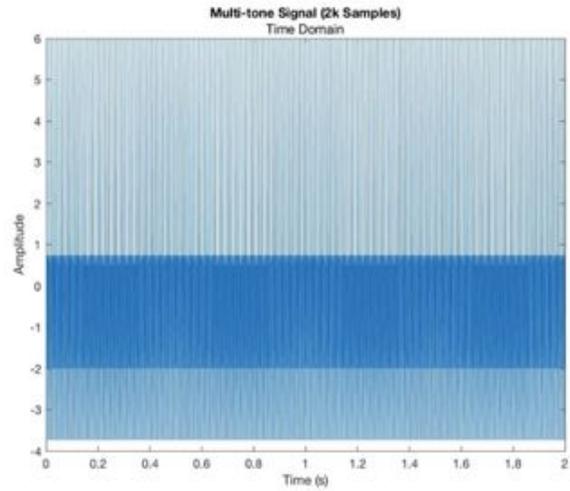


Figure 2. Theoretical plot of the frequency spectrum of the multi-tone signal.

MATLAB was used in order to apply these learned tone signal fundamentals. In MATLAB a multi-tone signal was created that consisted of three frequency components ( $f_1$ ,  $f_2$ ,  $f_3$ ). The signal was sampled at a 1000 & 2000 Hz sampling frequency and 1000 & 2000 samples were used. A plot of the time domain was generated (shown in Figure 3) and a fast Fourier transform was performed on the signal in order to get the frequency spectrum of the signal to also plot the signal in the frequency domain (shown in Figure 4).

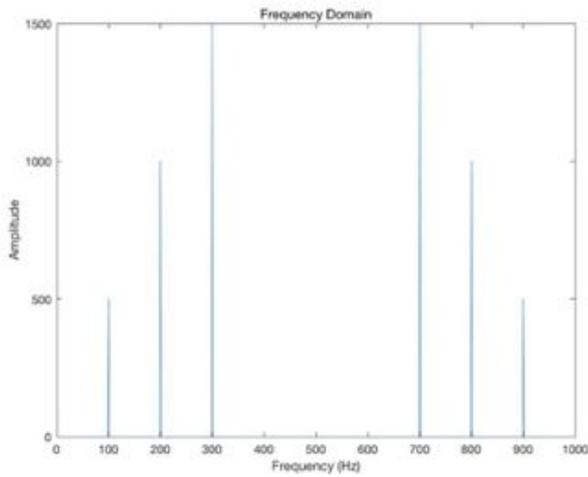


(a)

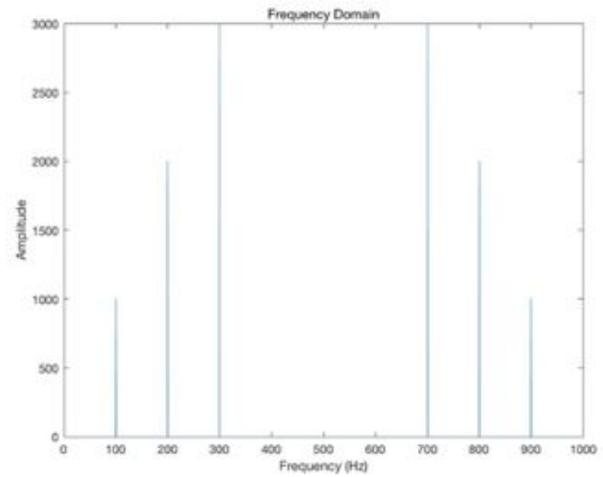


(b)

Figure 3 (a): Time domain of the multi-tone signal (1000 samples) (b): Time domain of the multi-tone signal (2000 samples).



(a)



(b)

Figure 4 (a): Frequency spectra of the multi-tone signal (1000 samples) (b): Frequency spectra of the multi-tone signal (2000 samples).

Additionally, MATLAB was used to apply a hanning window to the multi-tone signal in the frequency domain and a frequency spectrum plot was generated in order to visualize the effect of windowing on the amplitudes of each frequency component (shown in Figure 5).

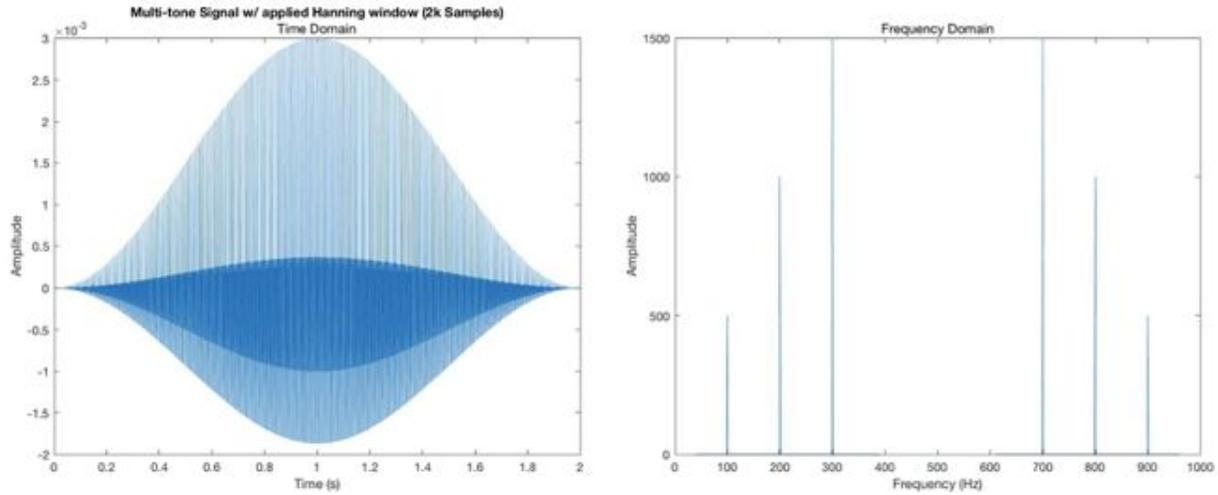


Figure 5 (a): Time domain of the windowed multi-tone signal (hanning window) (b): Frequency domain of the windowed multi-tone signal (hanning window).

The magnitudes of each frequency component ascend left to right from low to high. Furthermore, there is a difference between the magnitude and the original amplitude of the individual tones (the magnitudes appear to be  $\frac{1}{2}$  the product of N number of samples and the original amplitude. In the frequency domain, each tone signal can be easily distinguished compared to the respective time domain representation. The frequency domain representation allows one to see impulses of each tonal signal at their respective frequencies, while the time domain shows a summation of each tone signal which creates a messy signal that cannot be analyzed as easily as the frequency domain representation. The frequency domain of the multi-tone signal of 2000 samples shows the same magnitudes as the previous signal of 1000

samples. Again, there is a difference between the magnitude and the original amplitude of each tone signal, which is  $\frac{1}{2}$  the product of N number of samples and the original amplitude. The Fast Fourier Transform reduces the amplitudes of a given multi-tonal signal's frequency components due to the application of the rectangular window. The magnitude of this reduction varies based on the N number of samples of the signal, which directly correlates to the window length. Amplitudes must be fixed by dividing the transformed signal by the length of the signal. Furthermore, the amplitudes must be normalized by multiplying its values by the microphone responsivity.

The code used in MATLAB was translated into the python coding language. Additionally, the amplitudes of the 3 frequency components in the frequency domain were converted from volts (V) to db SPL using a given microphone responsivity. Finally, after the investigation of the fundamentals of tone signals and the application of those fundamentals in MATLAB and python, the multi-tone calibration was ready to be implemented into the existing PyVib2 code. PyVib2 is used to analyze vibrational motion and vibrational spectra. The code was implemented by simply integrating a for loop into the PyVib2 code that produced an individual pure tone stimulus. Furthermore, the existing code needed to be adjusted in order to accommodate for the new for loop. Specifically, the existing plotting code and *spCal* function needed to be integrated into the new for loop in order for the new multi-tone calibration implementation to work and display the calculated information properly.

The hardware configuration (shown in Figure 6) began with the PyVib2. Upon running the program, the digital multi-tone signal (which consisted of 4, 5, and 6 kHz pure tones) was converted to electrical signals using a DAQ, reduced by an attenuator, and magnified by an

amplifier. The electrical signals were used to output the multi-tone signal from a speaker, which then was recorded by a microphone. Then the signal recorded by the microphone was sent back to the DAQ and was read by the PyVib2 program in order to generate the following plots (shown in Figure 7) for further analysis.

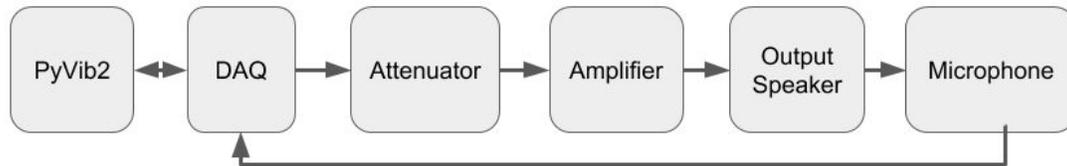


Figure 6. Hardware configuration for the multi-tone complex calibration.

For the second part of the experiment, a handheld OCT device was used in conjunction with the PyVib2 program to collect an M-Scan of the inner-ear while stimulating its structures by simultaneously outputting a multi-tone stimulus. In order to generate a multi-tone frequency response, the PyVib2 program parameters were set to play five single tones simultaneously from the built-in speaker on the handheld OCT device. These five single tones were set to 250 Hz, 500 Hz, 1 kHz, and 2 kHz.

## CHAPTER III

### RESULTS

Once the PyVib2 multi-tone calibration was implemented and the hardware configuration was completed, the PyVib2 program was used to collect data in order to validate the success of this implementation. The results of the multi-tone calibration are shown in Figure 7.

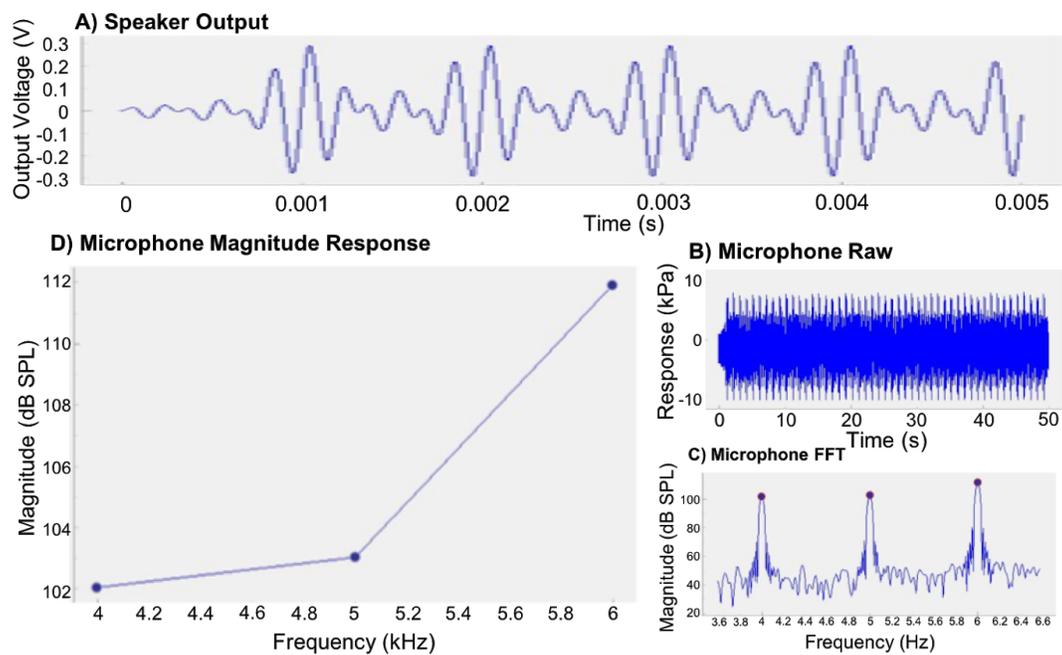


Figure 7. Speaker Output: shows the outputted multi-tone signal generated using the PyVib2 program. B) Microphone Raw: shows the received multi-tone signal recorded by the microphone. C) Microphone FFT: shows the computed frequency spectra of the raw mic data, which was used to confirm the input signal matched the output signal. D) Microphone Magnitude Response: shows a closer view of the respective frequency components of the multi-tone signal (4, 5, and 6 kHz) and their respective amplitudes (102, 103, 112 dB SPL).

Both the Microphone FFT and Microphone Magnitude response plots (shown in Figure 7, C and D) reveal that the program successfully output the desired multi-tone signal. Furthermore, the program successfully identified the peak amplitudes of each frequency component and their respective amplitudes 102, 103, and 112 dB SPL (shown in Figure 7, D) Microphone Magnitude Response).

After verifying the functionality of the PyVib2 multitone calibration with the provided hardware configuration, OCT imaging was now able to be conducted. In order to collect an in-vivo M-Scan of the human tympanic membrane, malleus, incus, and stapes the handheld OCT device was inserted into the ear canal of our human subject and stabilized. After the device was inserted and stabilized, the position of the device was adjusted so that the structures of interest (tympanic membrane, malleus, incus, & stapes) were centered in collection area of the PyVib2 program, which could be viewed using the program's real-time image display. Once the position of the device was determined and set, an M-Scan was collected while the multi-tone stimulus was outputted by the speaker on the handheld OCT device. During this collection period, it was important that our subject was stabilized so that any movement was minimized during the M-Scan collection process. The resultant M-Scan produced a BM-Scan (shown in Figure 8) that depicts two structures of interest (tympanic membrane and malleus).

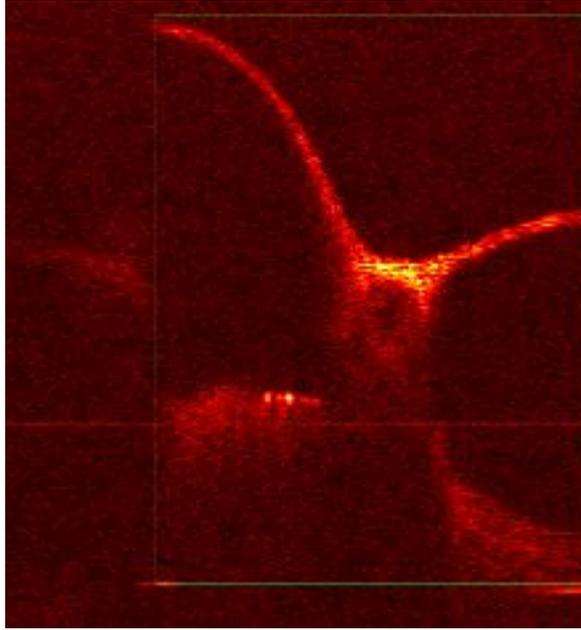


Figure 8. In-vivo OCT B-Scan of a human tympanic membrane and malleus acquired from the OCT M-Scan using the PyVib2 program.

After the collection of the raw M-Scan data from the PyVib2 program, MATLAB was used for analysis. The raw M-Scan data was processed in order to obtain the vibrometric data of the tympanic membrane. The raw M-Scan data consisted of 57 BM-scans, 5000 a-lines, and the image dimensions were 2048x2048 pixels.

After obtaining the vibrometric data, the phase response of the tympanic membrane in the frequency domain was extracted from the data structure and analyzed at different locations along the tympanic membrane. The reference for these locations can be seen in Figure 9, which is the average image calculated in MATLAB using the 57 BM-scans.

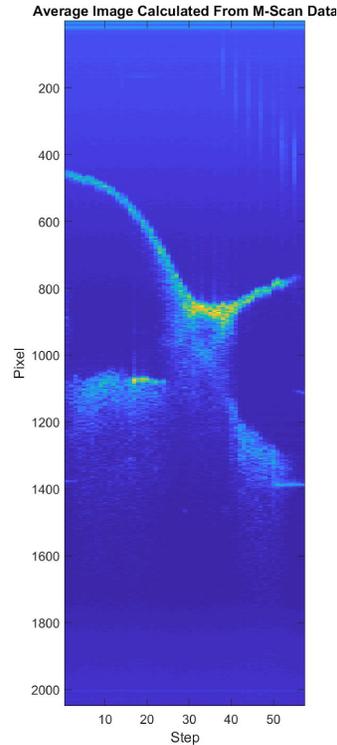


Figure 9. Average image calculated in MATLAB using the 57 BM-scans extracted from the processed raw M-Scan data. This average image was used to find specific locations along the tympanic membrane that exhibited a strong OCT signal.

Using the average image, locations that exhibited a strong OCT signal were found to be at Pixel 853/Step 33, Pixel 872/Step 37, and Pixel 889/Step 38. The frequency analyses at these areas on the tympanic membrane (shown in Figure 10) revealed peaks at 500 Hz, 750 Hz (a harmonic of 250 Hz), 1 kHz, and 2 kHz, which corresponds to the tones played from the speaker using the PyVib2 program. A possible reason for no distinguishable peak at 250 Hz was that the motion was too low at that frequency with the given noise floor; however, the other tones outputted by the PyVib2 program and the harmonic and 750 Hz are easily distinguishable.

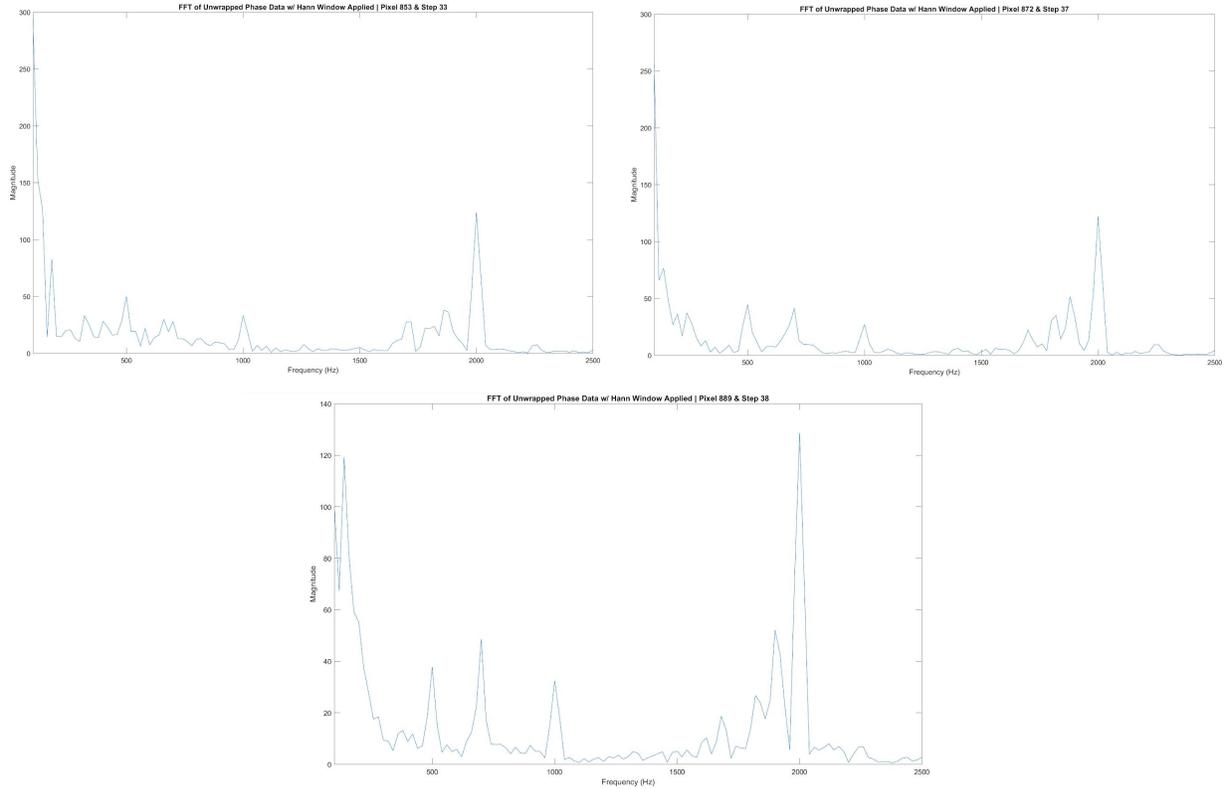


Figure 10. Frequency analyses of the phase response extracted from different locations on the tympanic membrane that exhibited a strong OCT signal. These locations include: Pixel 853/Step 33, Pixel 872/Step 37, and Pixel 889/Step 38, respectively.

After verifying that the locations on the tympanic membrane vibrated at these expected frequencies, the specific indices of each tonal frequency played from the speaker (500 Hz, 750 Hz [harmonic of 250 Hz], 1 kHz, and 2 kHz) were used to extract the spatial information at these individual frequencies in order to create four vibrational images for further analyses (shown in Figure 11).

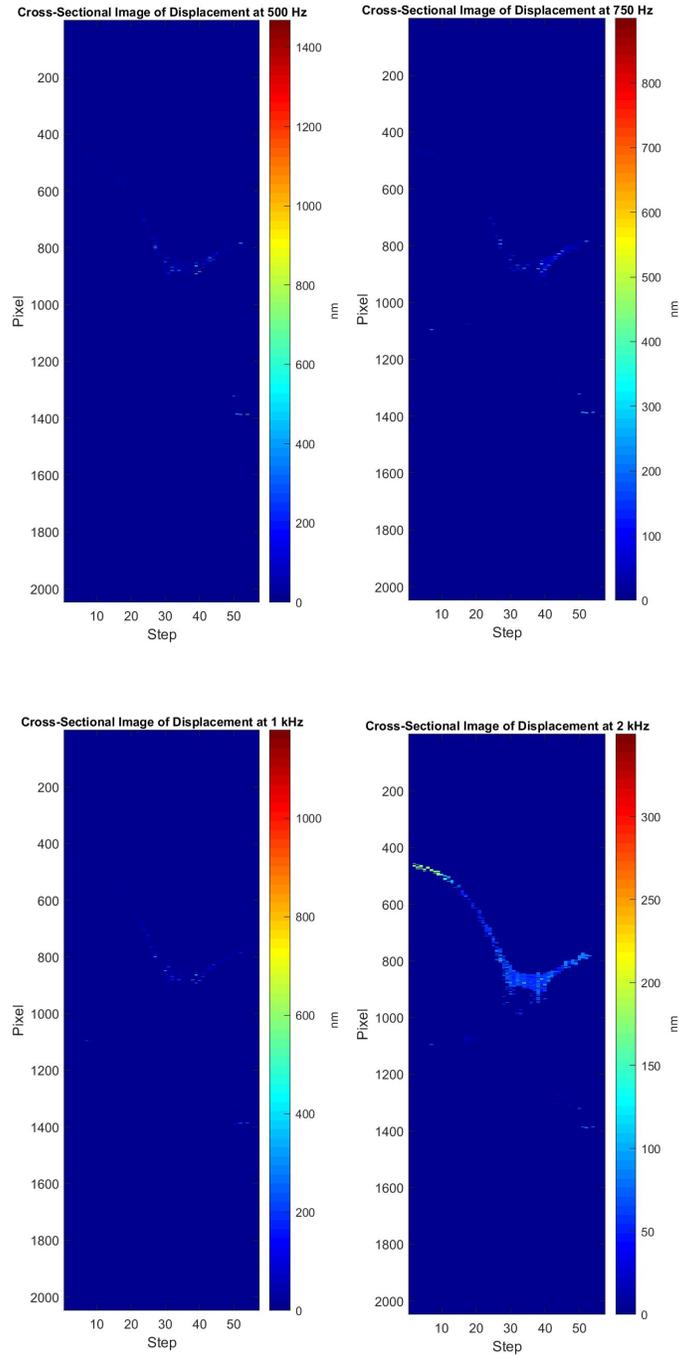


Figure 11. Four vibrational images showing the dynamic range of vibration across the tympanic membrane for each tone outputted by the PyVib2 program during the M-Scan collection (500 Hz, 750 Hz [250 Hz harmonic], 1 kHz, and 2 kHz).

## **CHAPTER IV**

### **CONCLUSION**

In this study, the fundamentals of multi-tone signals in both MATLAB and Python were evaluated in order to gain a better understanding of the various concepts involved with multi-tone calibration. These concepts included pure tone signals, multi-tone signals, frequency spectra of multi-tone signals, effects of windowing, and magnitude of frequency amplitudes based on window length. The presented data shows the successful implementation of multi-tone calibration into the PyVib2 program.

Using the discussed concepts, the PyVib2 program, and the handheld OCT system, the analysis of an in-vivo M-Scan of the human tympanic membrane was verified to provide vibrometric data that revealed the frequencies at which this inner-ear structure was vibrating at the time of the M-scan collection. A limitation of using this method for frequency analyses is that in order to collect the raw OCT data, the patient must be completely stabilized and motionless for a few seconds. The complete stabilization of the patient is a very difficult task to accomplish, even in a very controlled environment, due to slight movements such as the expanding and contracting of the lungs as the patient breathes. Despite this limitation, the information gained from these analyses could be used in audiometry to quantify hearing loss as well as generate a frequency envelope that could be used in the specific tuning of hearing aids for the patient. Therefore, this new method decreases the amount of time a patient must spend in the audiometry room from 30 minutes to a few seconds.

## REFERENCES

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