

Adapting a Commercial Spectral Domain Optical Coherence Tomography System for Time-locked Displacement and Physiological Measurements

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Abstract. Measuring vibration within the cochlear partition is important to understand sound transduction and nonlinear amplification within the cochlea. Here we describe the customization of a commercially available Spectral Domain Optical Coherence Tomography system, the Thorlabs Telesto, for displacement measurements in the cochlea using a technique called Spectral Domain Phase Microscopy (SDPM). The paper describes the SDOCT data analysis pipeline composed of background subtraction, interpolation, and spectral shaping of the raw photodetector data, followed by the implementation of SDPM for vibration measurements. It also details the software and hardware modification necessary to couple the Telesto to a Tucker-Davis Technologies data acquisition system to obtain time-locked vibration and electrophysiological measurements from the gerbil cochlea. The use of this commercial imaging system for auditory displacement measurements brings this powerful measurement tool within reach of many auditory researchers.

INTRODUCTION

Spectral Domain Optical Coherence Tomography (SDOCT) is a low-coherence interferometric technique used mainly for imaging. SDOCT has steep optical sectioning based on its broadband light source, and a penetration depth of several mm resulting from working in the infrared range [1]. It is also useful for displacement measurements, using its functional extension, Spectral Domain Phase Microscopy (SDPM). SDPM is capable of simultaneous vibration measurements from all locations in the axial (viewing) direction [2]. In contrast, the standard method of cochlear vibrometry, heterodyne interferometry, is limited to measurements from one location at a time, and cannot measure from deep within the tissue. Several auditory groups have developed and used bench-top SDOCT systems to observe inner ear vibration [3, 4, 5]. Our goal was to customize a commercially available SDOCT system for vibration measurements. A brief summary of how SDOCT works, and is used to measure vibration follows: The light source is split into object and reference beams and the object beam illuminates the sample. Light returning from reflectors along the axial illumination line is combined with the reference beam, interfering either constructively or destructively for different wavelengths in the light source. This combined beam is sent through a spectrometer, which separates the light by wavelength, to a linear photodetector array. The pattern of light along the photodetector array is termed an interferogram. Analysis of this wavelength-domain interferogram (described in more detail below) recasts the interferogram pattern into the spatial domain. The amplitude of the spatial domain function, referred to as an A-scan, has peaks at locations representing sample reflectors (Fig 1D). The displacement of the reflectors along the A-scan is found by analyzing the time dependence of the phase of the spatial domain function. To find the displacement, the phase is multiplied by $\lambda_0/4\pi n$ where λ_0 is the central wavelength of the broadband light source and n is the refractive index of the medium [2, 6, 7].

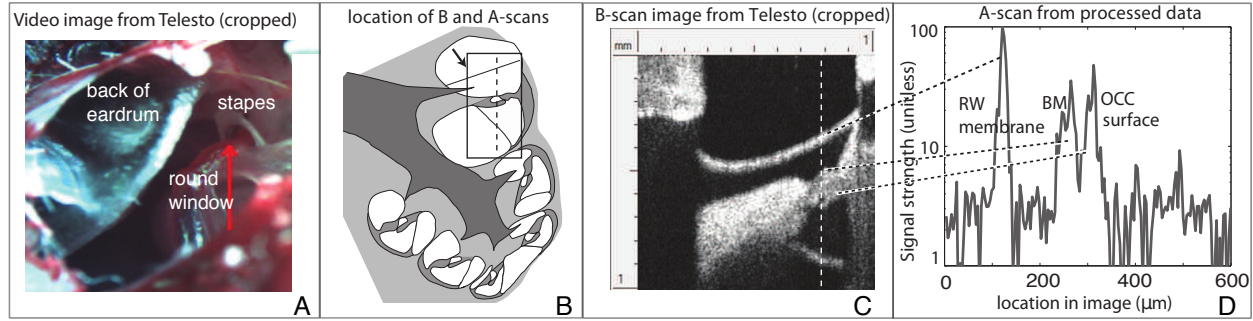


FIGURE 1. (A) Still image from the Telesto video camera, showing the view to the basal region of the gerbil cochlea. The arrow in A, drawn with the Telesto GUI, chooses the lateral axis of the B-scan. (B) Cartoon of the view. (C) Corresponding B-scan, with round window (RW) membrane, basilar membrane (BM) and organ of corti complex (OCC) surface in view. The black and white dashed lines in B and C give the location of the A-scan in D. The black and white dashed lines from D to C link the prominent peaks in the A-scan with the corresponding structures in the B-scan.

METHODS

Thorlabs OCT file processing steps and functions

We took two routes to use the Thorlabs Telesto III for vibration data acquisition, one using the Thorlabs GUI, ThorImage, and the other using the Thorlabs C++ Software Development Kit (SDK). ThorImage is designed for imaging. In its standard mode, A-scans are continuously acquired while scanning laterally with mirrors, resulting in a 2-dimensional B-scan. By not moving the mirrors, the B-scan becomes a single A-scan, resampled continuously in time, and is called an M-scan. This can be implemented with ThorImage. The raw data in an M-scan can be processed to measure the displacement of all the points within the A-scan. We recommend becoming familiar with data acquisition with ThorImage before using the Thorlabs C++-SDK. However, for streamlined data acquisition, the C++-SDK or something similar must be used. With the C++-SDK, data acquisition can be automated to allow for looping through stimulus parameters like frequency and sound pressure level. Also, when using ThorImage, a maximum of 10000 continuous A-scans can be taken but with the C++ SDK the limit is much higher. For example, we typically take 51 repetitions of 2048 A-scans, in a stimulus set (one frequency and one amplitude).

During every data acquisition scan (whether using ThorImage or C++-SDK) the Telesto first illuminates the photodetectors with the reference beam alone and takes a Thorlabs-set number of preliminary shots. These measure the source spectrum, and serve as background for data normalization. Processing of the data, schematized in Fig. 2, is similar whether the GUI or the C++-SDK are used for data acquisition. However, there are GUI/C++-SDK -dependent differences. For one thing, informational files needed to process the data are accessed differently depending on whether the GUI or the C++-SDK was used for data acquisition. These are the “electron scaling constant”, the “offset error” and the “chirp function”, used for interpolating the raw data from the wavelength-domain to the k-domain. If ThorImage was used for acquisition, each scan generates a directory that contains the files associated with the scan, including the background (spectrum) data, the scan data, and the three processing files referred to above. Matlab functions provided by Thorlabs can be used to open and process the ThorImage-generated files. Alternatively, to use the C++-SDK, Thorlabs provides drivers for initializing the system, setting the scan parameters, and recording of data. There are several example programs inside the SpectralRadarDemo.cpp that Thorlabs provides, and these can be used as a reference to set up the C++-SDK. When using the C++-SDK, “offset error” data is saved during the scan initialization process with a C++ command; the chirp function, which is machine-dependent but unchanging, can be copied from a ThorImage-generated directory and then stored in a permanent folder for repeated use. The electron scaling constant is also unchanging. Even when using the C++-SDK for acquisition, ThorImage is used to set up the view. The easy switching between video camera, B-scan and A-scan images greatly facilitates orienting the approach (Fig. 1). Whether the data were acquired with the GUI or the C++-SDK, investigator-written Matlab scripts are used to go through the processing steps outlined in Fig. 2.

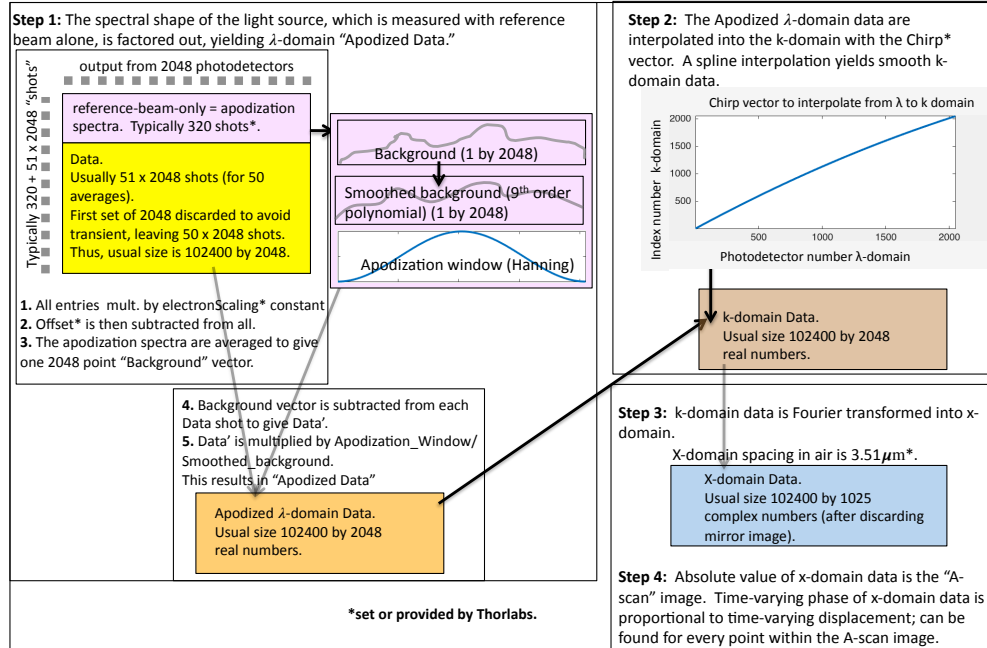


FIGURE 2. Flow diagram for processing raw photodetector data to obtain the complex A-scans. The diagram is appropriate whether the GUI or the SDK was used for acquisition, except for the number of shots, which for the GUI is at most 10000. In step 1 raw photodetector data is scaled, background-subtracted, and windowed to get the apodized λ -domain data. In the upper left panel of the diagram, the raw OCT data are represented schematically as a matrix, the rows of which represent the 2048 photodetectors and the columns the 104448 continuous A-scans. In step 2 the λ -domain data is interpolated to the k-domain, and in step 3 it is Fourier transformed to get the complex-numbered x-domain data. The amplitude of the x-domain data is an axial image (A-scan). The time variation of the phase of the x-domain data is used to find displacement of any point in the A-scan.

External triggering of the Telesto III with the TDT system

The Thorlabs Telesto III can be externally triggered by a high-duty-cycle TTL pulse train. We created a 100 kHz high-duty cycle pulse train using the Tucker-Davis Technologies (TDT) Real time Processor (RPvdsEx) and a purpose-built hardware circuit. Logic processing within the TDT RPvdsEx was used to turn an internal trigger pulse into a 50 kHz pulse train, which was then processed by the hardware circuit to generate the needed 100 kHz high-duty-cycle pulse train. The schematic is shown in Figure 3.

The TDT RPvdsEx circuit works as follows: A one-shot and a trigger pulse are input into an RS flip-flop (trigger signal at set, one-shot at reset); the function of the one-shot is to reset the memory of the RS flip-flop ($Q=0$) with an impulse. The rising edge of trigger signal ($R=0$ $S=1$) generates a step-function $S1$. $S1$ is input into both J and K of a JK flip-flop. This toggles its output Q (since $J=1$ $K=1$) at the sampling rate of the TDT (100 kHz). This produces a pulse train T1 with twice the sampling period, so at 50 kHz.

Since the Telesto III requires a TTL pulse train that has a high-duty-cycle, T1 needs to be modified. In addition, we need to restore the 100 kHz sampling rate so that the OCT will be fully synchronized with the TDT. For that we constructed a hardware circuit. A purpose-built rising and falling edge detector was created with four XOR gates on a CD4077BE chip. T1 is input into three grounded XOR gates to obtain a nanoseconds time-delay. By inputting T1 and the time-delayed T1 into the fourth XOR gate, impulses are generated at both the rising and falling edges of T1. When inputting the impulses into a non-retriggerable monostable multivibrator (DM74LS221N), a pulse train with an $\sim 8 \mu\text{s}$ high and $\sim 2 \mu\text{s}$ low is produced to trigger the Telesto III.

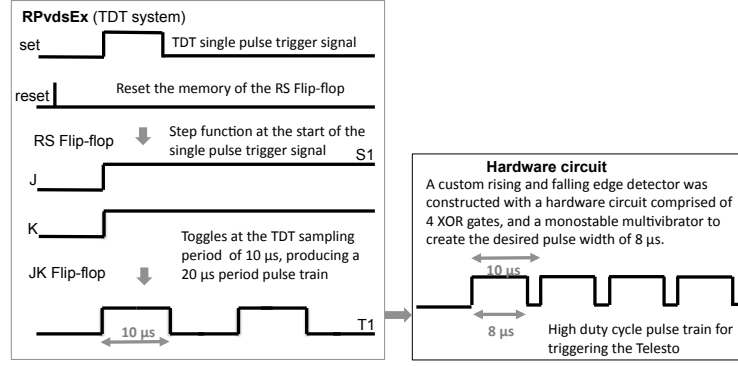


FIGURE 3. Schematic of the generation of a high-duty-cycle pulse train for the Telesto from the TDT system. The single trigger pulse from the TDT is used to create two identical step functions (with an RS Flip-flop). These were then toggled by the TDT 100 kHz sampling rate to create a 10 μ s period pulse train (with a JK Flip-flop). Lastly, a rising and falling edge detector and a nonretriggerable monostable multivibrator were used to create a high-duty cycle pulse train.

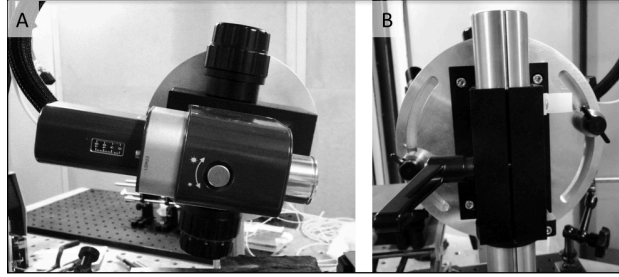


FIGURE 4. Telesto rotation mechanism. A set of circular aluminum plates with arced cut-outs and intra-plate locking is connected between the probe head and the vertical mounting rod. (A) Front view of Telesto probe, angled almost horizontally. (B) Back view, showing arced cut-outs and locking screws. (Designed and machined by the CUMC machine shop, under Gary Johnson.)

RESULTS

LDV comparison

To verify that our triggering system was working without jitter and to verify the accuracy of the displacement measurements, we compared the response of the Telesto to that of our Polytec laser Doppler velocimeter (LDV). The vibrometer measures velocity directly and for the amplitude comparison, the velocity has been converted to displacement, using $x = v/2\pi f$. For this test, the displacements of a piezoelectric actuator and the driving voltage to the actuator were simultaneously recorded by the TDT to give a synchronized reference for the phase. In the first experiment, we applied increasing magnitudes of 10 kHz sinusoidal driving voltage. The LDV response we observed verified the linearity of the actuator's displacement. The OCT displacement measurement was consistent with that of the LDV (Fig. 5A). In the second experiment, a 3 V sinusoidal driving voltage with frequencies ranging from 100 Hz to 5 kHz was sent to the actuator. After accounting for the instrument delay (mainly from the Telesto since it takes a set number of background scans before data acquisition), the LDV's phase led the SDOCT's phase displacement by one-quarter cycle across all frequencies (Fig. 5B). This is as expected since the LDV measures velocity and the OCT displacement.

In vivo measurement

Data from an *in vivo* gerbil measurement, taken from a location similar to that of Fig. 1, are shown in Fig. 6. Data were recorded at two sound pressure levels: 70 and 90 dB. The basilar membrane (BM) responses were weakly nonlinear in this preparation. The BM and putative reticular lamina (RL) position were identified from the B- and A-scans and

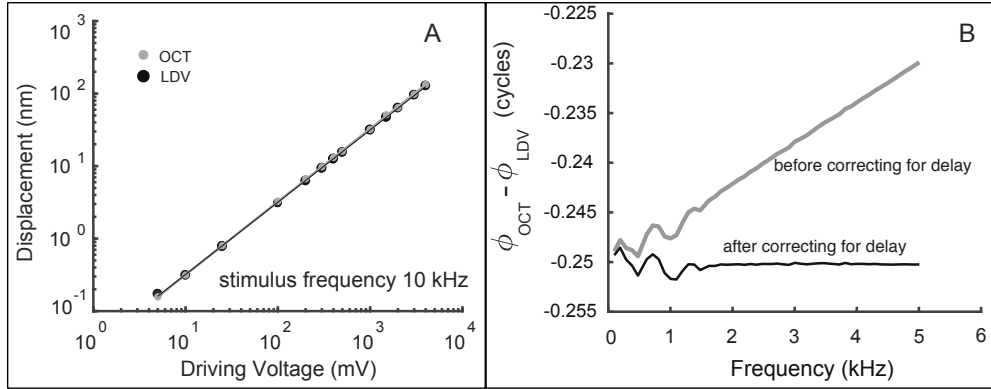


FIGURE 5. (A) The displacements of a piezoelectric actuator over a range of driving voltages were measured by both the LDV and SDOCT systems to verify the consistency. B. A frequency sweep was done on the same actuator at a fixed driving voltage to look at the phase delay between the LDV and OCT systems. After correcting for the instrument delays, the two systems show a quarter cycle difference in their phases.

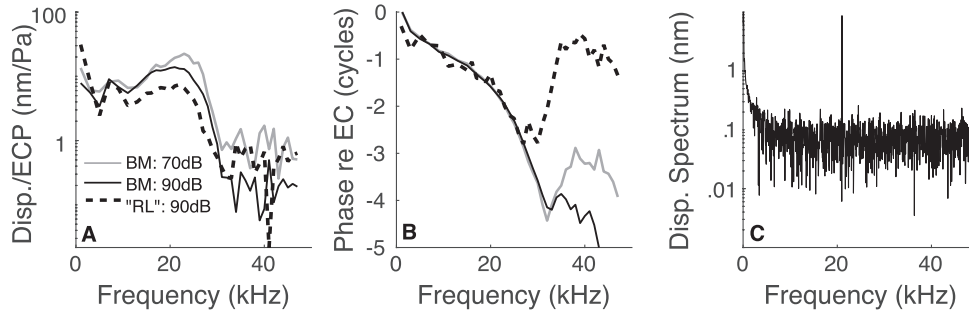


FIGURE 6. *In vivo* organ of Corti displacement measurements, with 70 and 90 dB SPL stimulation. A. Amplitude of the response at the BM and RL normalized by the sound pressure level. B. Phase relative to ear canal pressure, measured simultaneously. C. Displacement spectra from BM location at 90 dB and 20 kHz measurement, showing noise level.

the displacements at those locations are plotted. The RL peak in the A-scan was smaller than that of the BM, and this results in a higher noise level; thus we have only shown the RL response at the higher sound pressure level. Peaking and traveling wave phase accumulation were apparent on both structures. The spectra shown in C is from the BM position and shows a noise level of $\sim 0.2 \text{ nm}/\sqrt{\text{Hz}}$, flat with frequency above $\sim 4 \text{ kHz}$, and increasing at lower frequencies. These data were taken with our usual 1 second of data acquisition, after averaging 50 time-locked responses, each 2048 points long.

DISCUSSION

We modified a commercial optical coherence tomography system, the Thorlabs Telesto III, with software and hardware alterations, to acquire time-locked displacement measurements using Spectral Domain Phase Microscopy (SPDM). Because its light source operates in the infrared, OCT as implemented with the Telesto III is able to image structures within the intact cochlea and simultaneously record from multiple layers. The technique is thus of great interest to those studying auditory mechanics. The OCT system is frequency-locked to our TDT sound generation system and is able to record at a sample rate of 100 kHz, with sub-nanometer resolution. As a demonstration of the system, we measured the sinusoidal displacements of a piezoelectric actuator using the OCT and a commercial LDV and the two methods compare favorably. We have also tested the system with a number of gerbil preparations and have presented displacement data from an *in vivo* cochlea.

Although other groups have used OCT and SPDM to study vibrations in the intact cochlea [3, 4, 5], the majority

of these studies have used custom-built systems. The goal of this work was to develop a methodology with which auditory scientists without an extensive background in the construction of optical systems can use an SDOCT system for sub-nanometer scale displacement measurements. The Thorlabs Telesto III cost \$80,000. Our setup is implemented with minimal hardware modifications and is controlled using relatively simple custom software. We have described the steps to synchronize the OCT to Tucker Davis signal generation hardware, but the OCT can be controlled using any such system. Additionally, the raw data generated by the instrument can be processed into time waveforms using simple image processing scripts. These developments bring OCT imaging and SPDM to a larger number of research groups in the auditory mechanics field.

ACKNOWLEDGEMENTS

This work was funded by NIH grant R01-DC015362 and the Emil Capita Foundation.

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QUESTIONS AND ANSWERS AFTER THE PODIUM PRESENTATION

Question 1: One of the things we are doing with our LDV is called reflector optometry, what it does is that it uses the flight time of the laser, the change in the refractive index of the medium, that the laser is moving through. So when you get a sound pressure wave passing through air, if you let the laser go through it and bounces and comes back to you. You can actually measure the sound pressure wave. It is entirely possible that is what you are measuring here, in between the moving membrane and the stationary fiber. What you might be measuring is the pressure built up in the object, which changes the refractive index of the medium in it and that is what your pickup is.

Answer 1: We do not think so since phase noise is a known property in the optics field, and this is definitely a case of phase noise.

Question 2: Solution to the signal competition problem?

Answer 2: We can either have a super broadband light source, so that the axial resolution is better and the point-spread function is steeper, so the neighboring depth reflectors would not influence each other as much. Or we can make the sample more reflective so that it would be less prone to signal competition.

Question 3: Have you thought about using a weighted phase? Since your problem area is between the A-scan structures usually have lower intensity.

Answer: The solution is that we should always avoid the local minimums when getting the displacements. As long as you are on a local maximum, you are fine because of the steep optical sectioning.

Question 4: This is the same system that Marcel and Nigel are using, right? How come the picture doesn't look as good? What is the difference? Did they zoom in more?

Answer 4: They are using the same commercial system from Thorlabs.