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Multisine excitation enables frequency dense OCT-vibrometry for middle ear mechanics

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Multisine excitation enables frequency dense OCT-vibrometry for middle ear mechanics

Pieter Livens, Sam Van der Jeught, Adrian Bradu

Abstract— Optical coherence tomography (OCT) vibrometry enables non-invasive, high-resolution measurement of tissue motion with nanometer sensitivity. To our knowledge, this paper presents the first direct comparison of full-field modal shapes generated by pure and multisine acoustic stimulation tones in OCT vibrometry. Measurements were performed on two rubber membranes and two ex-vivo rabbit eardrums. Using multisine stimulation, we could identify a resonance of the rabbit eardrum at 1.4 kHz. The first eardrum had a displacement of 180 ± 38 nm/Pa at its center and the second eardrum 81 ± 17 nm/Pa at resonance, respectively. The pure tone measurements had similar displacement amplitudes, but lower standard deviations due to the lower uncertainty in pressure level. For the rubber membranes, the multisine data revealed distinct frequency responses. The first membrane had a resonance at 0.6 kHz and displacement of 3768 ± 1467 nm/Pa at its center, while the second membrane had a resonance at 1.2 kHz and displacement of 1718 ± 665 nm/Pa, respectively. While pure tones provided higher signal-to-noise ratios, multisine stimulation enabled rapid, broadband acquisition with comparable spatial detail. These results support a hybrid strategy in clinical OCT vibrometry: fast multisine screening followed by targeted pure tone analysis to enhance diagnostic precision.

I. INTRODUCTION

Optical coherence tomography (OCT) is a non-invasive, non-contact volumetric imaging modality that provides high-resolution volumetric data based on low-coherence interferometry [1]. Structural information is derived from the intensity of backscattered light, while phase information can also be retrieved, enabling interferometric displacement measurements with nanometer-scale sensitivity [2], [3].

The ability of OCT to collect structural and vibrational information has led to great interest in the field of hearing research. OCT can be used to assess structural pathologies such as middle ear effusion [4] or fixation of the ossicles [5]. Middle ear pathologies alter the frequency-dependent motion of the tympanic membrane and ossicles [6], [7], [8], [9]. Therefore, capturing vibratory responses across a wide acoustic spectrum could provide clinically relevant insights and aid in surgical planning.

One of the earliest demonstrations of OCT vibrometry on the human eardrum was reported in 2012 [10], and subsequent studies have visualized middle ear vibrations in humans, chinchillas, and mice [11], [12], [13]. Traditionally, pure tone sound waves are used when performing vibratory studies on the middle ear ossicles and eardrum [6], [8], [14], [15], [16]. Pure tones are easy to define and provide high signal-to-noise ratios. As an alternative, chirp signals [11], [17] have also been explored in OCT vibrometry. A chirp signal's frequency varies over a specific time interval. This allows for testing a system's response across a range of frequencies. However, they exhibit poorer signal-to-noise ratio measurements compared with when pure tones are employed, and require more dedicated signal processing [17]. Moreover, chirp signals contain transient effects, which makes direct comparison with pure tone data more difficult.

An alternative approach to broadband stimulation is the use of multisine (or multitone) signals, constructed as the sum of discrete pure tones. These stimuli offer control over both the number and distribution of frequencies, allowing targeted excitation while preserving high SNR [6], [18], [19]. Multisine signals are particularly attractive for efficient vibrometric assessments, but they remain underexplored in OCT-based middle ear studies. To our knowledge, only one previous study has applied multisine excitation in this context [17], and no systematic comparison of displacement patterns or modal shapes between multisine and pure tone excitation has been reported.

In this study, the first direct experimental comparison of pure tone and multisine stimulation in OCT vibrometry is presented. Using a custom-built setup, the vibrational responses of two rubber membranes and two intact rabbit middle ears under both excitation schemes were measured. The aim was to assess differences in displacement magnitude, modal shape visualization, and practical considerations such as signal-to-noise ratio and measurement efficiency.

These insights may contribute to the development of more rapid and robust diagnostic tools for future clinical implementation of OCT vibrometry.

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II. METHODOLOGY

Pure tone versus multisine stimulation

When using spectral domain OCT setups, as is the case in this work, reflectivity profiles (A-scans) are typically computed by calculating the Fourier transform of the channeled spectra produced by mixing the light backscattered by the sample and from the reference arm of the interferometer. As a result, the A-scan contains amplitude and phase information from any point inside the sample investigated. Displacement of scatterers within a medium will cause changes in the phase of the OCT signal. The phase difference $\Delta\Phi(t)$ between consecutive A-scans can be related to the displacement of the scatterer $\Delta z(t)$ as [1], [2], [20]

$$\Delta z(t) = \frac{\lambda_c}{4\pi} \Delta\Phi(t) \quad (1)$$

In the equation above, λ_c denotes the central wavelength of the optical source that the OCT instrument is equipped with. When the motion is a pure tone sine wave, one can write

$$\Delta\Phi_{\text{pure tone}}(t) = A \sin(2\pi\nu t + \theta) + \varphi \quad (2)$$

In Eq. (2), A denotes the displacement amplitude, ν the frequency of sound and θ the phase of the sound relative to the motion of the sample. The random phase noise determined by the optical setup is denoted by φ , and defines the noise floor of the measurement. To compute Δz , a sequence of A-scans is collected at a fixed location to determine the amplitude in Eq. (5). This is then repeated for the entire imaging volume.

To improve the collection of OCT-vibrometry data, the use of so-called multisine stimulation was explored, the idea being to define an input pressure signal $P(t)$, which is a linear combination of K sinusoidal pressures, containing frequencies ν_i and phases γ_i , respectively

$$P(t) = \frac{p}{\sqrt{K}} \sum_{i=1}^K \sin(2\pi\nu_i t + \gamma_i) \quad (3)$$

In Eq. (3), the total pressure level of the wave is p , and is normalized by $\sqrt{K}$ to keep the total sound intensity constant. The sound's phase γ_i is determined by minimization of the so-called crest factor using Matlab's *fminsearch* [18], [19], [21]. In doing so, large peak amplitudes in the signal $P(t)$ are avoided.

By construction, each frequency contributes equally to the motion of the sample with input amplitude $p/\sqrt{K}$, as illustrated by Eq. (3). The phase difference of the multisine $\Delta\Phi_{\text{multisine}}(t)$ can then be expressed as

$$\Delta\Phi_{\text{multisine}}(t) = \sum_{i=1}^K A_i \sin(2\pi\nu_i t + \theta_i) + \varphi \quad (4)$$

From one dataset, the motion of the amplitude A_i at corresponding frequency ν_i can then be constructed, which allows for direct inspection of the frequency response from one dataset.

Sample preparation

A patch of nitril rubber (VWR, model 112-2372) was stretched across a hollow metal cylinder with a 5 mm radius, similar to the one presented in [3]. The membrane was fixed using a metal washer bolted to the cylinder's top. Two openings were made along the cylinder's length: one opening to connect an earbud (Sennheiser, CX 300 II) for sound stimulation, and a second opening allowed the probe microphone (Brüel & Kjær, type 4182) to record the acoustic pressure. An illustration of the geometry of this sample is presented in the photo shown in Fig. 1A. The cylinder was fixed on a translation stage using double-sided tape and placed underneath the OCT imaging head. We created two rubber membrane samples in this manner. The first membrane was only slightly stretched when placed on the cylinder. In the second sample, the membrane was stretched further to determine if the multisine approach allows for discerning between different stiffnesses.

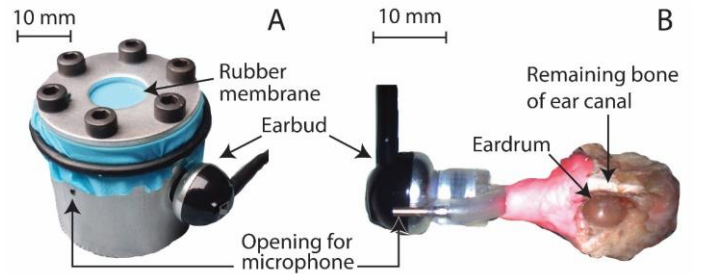


Fig. 1. A rubber membrane is placed on top of a hollow cylinder with 5 mm radius (A). An earbud allows sound stimulation from the membrane's back, and the sound pressure level can be recorded via a microphone. The ear canal of the rabbit is removed, allowing for optical access (B). Sound can be played through the earbud and calibrated with the microphone via an opening in the middle ear cavity.

To collect eardrum data, two rabbit heads were obtained from a local breeder (Peeters – Verbaeten, Ranst, Belgium). The preparatory steps to remove the ear canal, allowing visual access to the eardrum, have been described elsewhere [22], [23], [24]. The middle ear chain was left intact, and only the bony parts of the ear canal wall limiting optical access were removed. To facilitate the stimulation of the eardrum, a surgical perforation was created in the cavity of the middle ear. A 3D-printed microphone and speaker were accurately positioned over the designed aperture and securely sealed using a two component silicone paste (Dreve Otoplastik, Otoform Ak X). An illustration of a typical rabbit sample is presented in Fig. 1B. Two samples using this method were produced, which we will refer to as R1 and R2.

Experimental OCT setup & data processing

To measure the vibratory motion under the incoming acoustic pressure, a custom spectral domain OCT-vibrometry setup was developed. The instrument uses a superluminescent diode as an optical source, with a central wavelength of 840 nm and a 50 nm spectral bandwidth (Superlum Broadlighter, model S840). The axial resolution, determined by this source, experimentally measured, was 5 μm (in biological tissue). In the sample arm,

two orthogonal galvanometer mirrors (Thorlabs, model GVS002) were used to scan the optical beam over the sample. The beam was focused onto the sample by a 54 mm focal length scan lens (Thorlabs, LSM04-BB). Light back-scattered by the sample was then recombined with the light from the reference arm of the interferometer and detected by an in-house devised spectrometer. The spectrometer includes a transmission diffraction grating from Wasatch Photonics with 1,200 lpm, which diffracts the optical beam over the pixels of a fast line camera (Teledyne e2v, model Octoplus USB). Positioning of the galvanometers and triggering of the line cameras is done via a multifunction I/O device (National Instruments, model PCIe-6321) and custom LabVIEW software. The acquisition rate of the camera was 100 kHz, i.e., every 10 μ s, an A-scan profile is collected. Complex OCT images were produced using the Master-Slave approach [25] and displayed in real-time.

A laptop connected to a terminal block (National Instruments, USB-6251) was used to calibrate the earbud speaker voltage to the required pressure level. Using a custom MATLAB application, either pure tones or multisine sound signals were calibrated. The calibration loop was terminated when the measured pressure level was within 1 dB of the desired pressure. For all measurements, 5 ms of data (500 phase samples) at each A-scan (x,y) position were collected. Therefore, any multiple of 200 Hz fits wholly within this measurement duration.

When imaging the rubber membranes, 94 dB, which corresponds to 1 Pa acoustic input pressure, was used for both the multisine and pure tone measurements. First, multisine data were collected, with a linear frequency spacing between 400 and 8000 Hz in steps of 200 Hz (39 frequencies in total). Using the multisine data, the mode shapes were examined and pure tone measurements were collected at a selection of frequencies.

When imaging the eardrum, an input acoustic pressure of 100 dB was used for both the multisine and pure tone stimuli. To further improve the signal-to-noise ratio, the frequency range of 800-6000 Hz (27 frequencies) was used for multisine stimulation. As before, a multisine dataset was collected first, and then the multisine visualization was employed to select relevant pure tone frequencies.

A single vibrometry B-scan contained 130 lateral, 200 axial, and 500 temporal samples. To scan the measurement volume, 50 B-scans were collected since this provided a good balance between measurement duration, data size, and spatial resolution. The total scan duration was 5 minutes per frequency. The field of view was the same for both the membrane and the eardrum data collection, and the corresponding voxel sizes for the X, Y, and Z coordinates were 89, 224, and 5 μ m, respectively. The OCT intensity and phase data were written to binary files for further processing in MATLAB (R2024a).

Within MATLAB, the intensity data of each B-scan was first averaged across the 500 temporal phase samples for each pixel. Stacking the vibrometry B-scans, a C-scan volume containing the morphology of the sample was created. The resulting intensity volume was then loaded into segmentation software

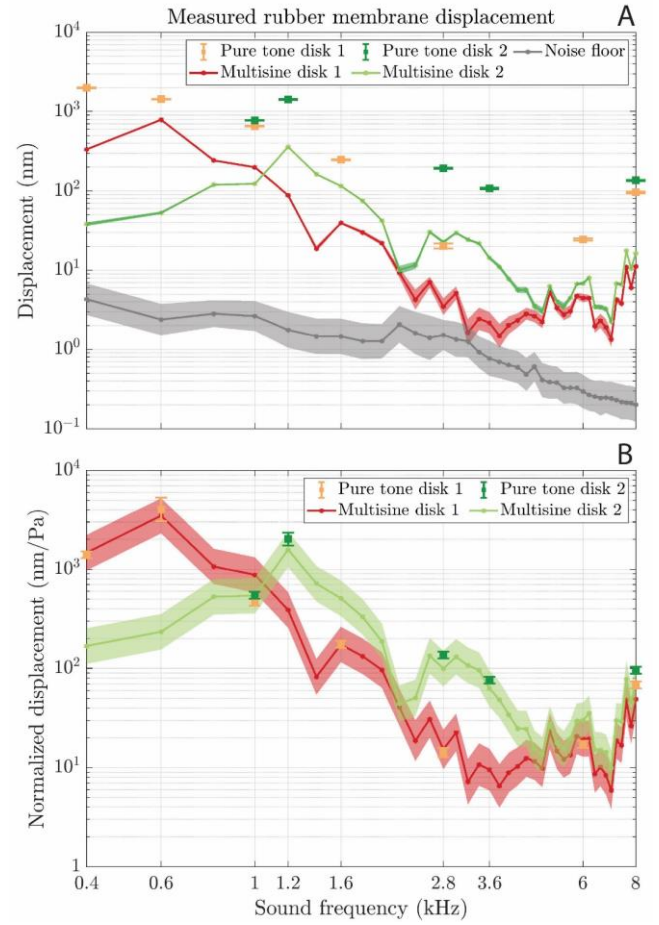


Fig. 2. Measured displacement amplitudes at the center of two disks (A). The spatial average is computed within a region of 0.5 by 0.5 mm². For pure tone measurements, the data is shown by square markers with corresponding error bars. The multisine data is shown by the solid line with shaded area. Normalizing by the input pressure level allows a clearer comparison of the pure tone and multisine data (B).

(Dragonfly 3D, V2024.1). Using Otsu thresholding and manual outlier removal, a mask was created separating the relevant voxels from background. Using Eqs. (1) and (4), the displacement amplitude is then computed by computing the Fourier transform of the differentiated phase data $\Delta\Phi(t)$.

III. RESULTS

Initially, the frequency response at the centers of the disks was investigated. Data was collected from the central area of each membrane within a 0.5 by 0.5 mm² square region. The mean displacement amplitudes computed at these locations are shown in Fig. 2A. All measurements were performed at 94 dB sound pressure. However, for disk 1, the displacement at 0.6 kHz under pure tone stimulation caused phase wrapping [20]. Therefore, this measurement was repeated at 82 dB, and the 94 dB data has been omitted. The pure tone data is shown by the square markers with corresponding error bars, while the multisine data is indicated by the solid lines with a shaded region, respectively. The OCT setup's noise floor is shown by the grey solid line, with the corresponding error margin.

To allow for better comparison of these data, the displacement amplitude normalized by the input pressure level was computed. The results are summarized in Fig. 2B. The corresponding error margins are computed via error propagation. The spatial average of the displacement at the center is denoted by $\hat{z}$. Then, the normalized displacement $\hat{z}_n$ becomes

$$\hat{z}_n = \frac{\hat{z} \sqrt{K}}{p} \quad (5)$$

with corresponding standard deviation σ_n

$$\sigma_n = \sqrt{\left(\frac{\sqrt{K}}{p}\right)^2 \sigma_z^2 + \left(\hat{z} \frac{\sqrt{K}}{p^2}\right)^2 \sigma_p^2} \quad (6)$$

In the Equation above, σ_z^2 is the spatial variance corresponding to $\hat{z}$ (shown in Fig. 2A) and σ_p^2 is the variance due to the uncertainty in pressure calibration, which is 1 dB SPL. The normalized data are presented in Figure 2B. A good match is found between the pure tone and multisine data. However, the pure tone data at 1 kHz is lower than expected. Since the use of the multisine excitation spreads the sound intensity over K frequency components, an increase in the error bounds is observed. However, the multisine data allows efficient inspection of the frequency response. For disk 1 (red curve with shaded area), a resonance at 0.6 kHz is evident. The displacement amplitude is 4044 ± 1110 nm/Pa for the pure tone measurement, and 3768 ± 1467 nm/Pa for the multisine data. For disk 2 (green curve with shaded area) the multisine data highlights a different resonance frequency at 1.2 kHz compared to disk 1. The amplitude is 2017 ± 295 nm/Pa for the pure tone data, while the multisine curves as an amplitude of 1718 ± 665 nm/Pa, respectively.

For the eardrums, the displacements at their centers were examined using the same procedure introduced above. Again, data were averaged within a 0.5 by 0.5 mm² region, see Fig. 3A. The normalized data are shown in Fig. 3B. Both eardrums show very similar frequency responses, with a resonance at 1.4 kHz. At the resonance frequency, the first eardrum has a displacement of 142 ± 22 nm/Pa for the pure tone measurement, and a displacement of 180 ± 38 nm/Pa for the multisine data, respectively. The second eardrum shows an amplitude of

around 64 ± 10 nm/Pa for the pure tone data, and an amplitude of 81 ± 17 nm/Pa, respectively.

After analyzing the frequency responses from the centers of the disks and eardrums, normalized displacement amplitudes across the surface of our samples were visualized. First, the segmentation mask was used to extract all relevant voxels, with their corresponding X, Y, and Z coordinates, from the volume. Then, finite element approximation is employed to improve data visualization, as described in [26], [27]. In essence, the nodal values of the mesh are computed by least-squares minimization of the displacement data within an element. In this way, all valid data is used, and the surface motion can be reconstructed. The results for a selection of frequencies are shown in Fig. 4. The top row shows the normalized displacements for pure tone stimulation, while the bottom row shows the results for multisine stimulation.

Comparing rows in Fig. 4, it can be observed that the multisine stimulation (bottom row) accurately captures the modal shapes measured using pure tone stimulation (top row). For disk 1, the resonance frequency was 600 Hz, and the typical shape of the

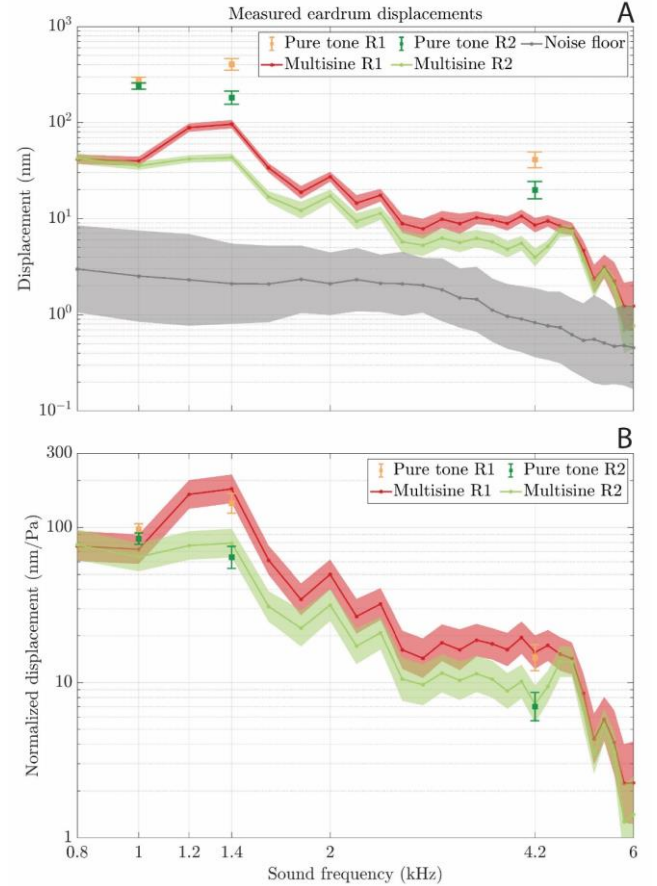


Fig. 3. Measured average displacement amplitudes at the center of the eardrums in a 0.5 by 0.5 mm² region (A). For pure tone measurements, the data is shown by square markers with corresponding error bars. The multisine data is shown by the solid line with shaded area. Normalizing by the input pressure level allows a clearer comparison of the pure tone and multisine data (B).

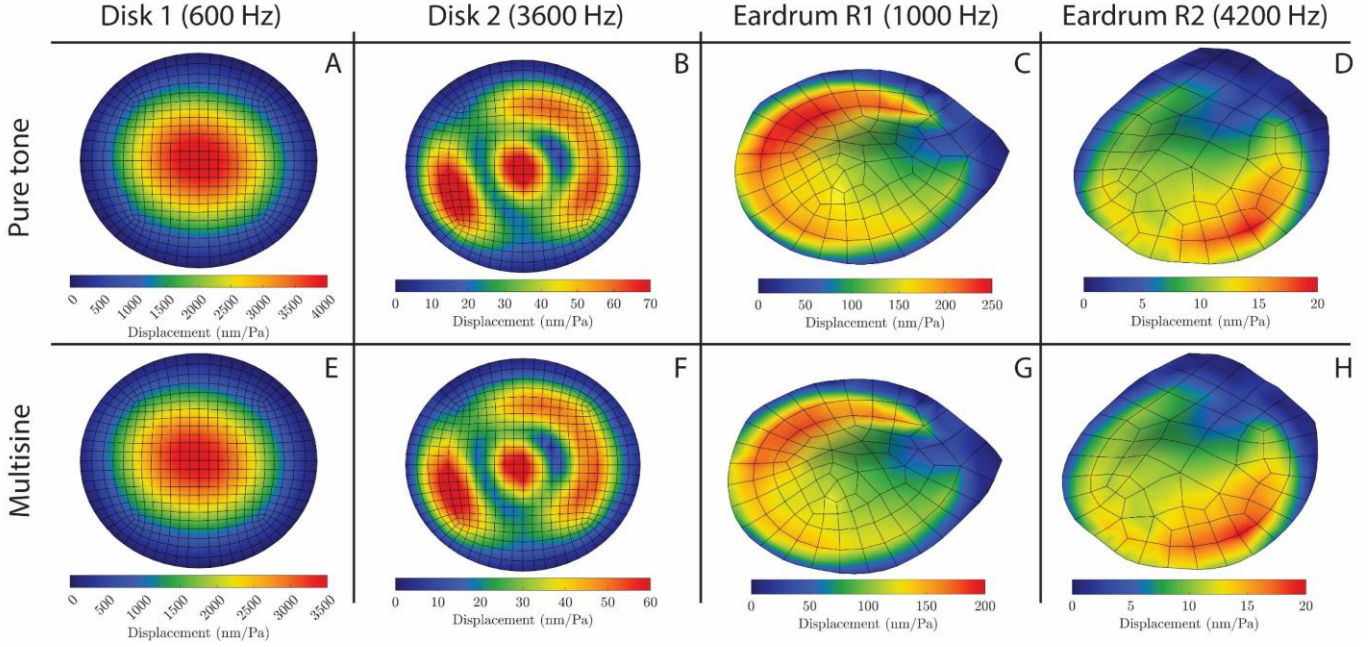


Fig. 4. Normalized displacement amplitudes using pure tone (top row), and multisine stimulation (bottom row). The OCT data has been interpolated to a finite element mesh to reconstruct the surface motion. For disk 1 (first column), we observe the first order mode at 600 Hz, which is accurately depicted by the pure tone (A) and multisine (E) datasets. For disk 2 (second column), a complicated mode shape is seen at 3600 Hz for the pure tone (B) and multisine data (F). Eardrum R1 has a resonance at 1000 Hz, visible both for the pure tone (C), and multisine (G) datasets. Eardrum R2 illustrates the displacement amplitude at 4200 Hz, for pure tone (D) and multisine stimulation (H).

first-order mode is observed, as illustrated in Fig. 4 (first column). For the second disk, the mode at 3600 Hz is visualized (second column). Again, the multisine and pure tone measurements are very similar. For the eardrums (third and fourth columns) a good correspondence between pure tone and multisine stimulation is evident. In Fig. A1 of the Appendix, all pure tone and multisine results for disk 2 are displayed, showing similar findings as in Fig. 4.

IV. DISCUSSION

In this paper, the first direct comparison of multisine and pure tone stimulation in the context of OCT vibrometry, specifically targeting the mechanical response of the tympanic membrane, is presented. Our results demonstrate that pure tone excitation enables clear identification of vibrational modes at discrete frequencies (see Figs. 2A and 3A). Because all acoustic energy is concentrated at a single frequency, normalizing the displacement by input pressure results in minimal amplification of uncertainty, as illustrated in Figs. 2B and 3B. While multisine excitation introduces broader uncertainty bounds, it offers a key advantage: the ability to characterize the frequency response in a single, time-efficient measurement. This trade-off between precision and measurement efficiency highlights the complementary roles of these two stimulation methods in middle ear vibrometry.

One assumption in our work is that the system response is linear for the pressure levels and frequencies explored in this paper. The Kirchhoff-Love linear deflection theory is valid when

displacement amplitudes are less than half the thickness [28], [29]. The rubber disks used in this study have a thickness of 100 μm , so the linear displacement limit is 50 μm . Since the maximal absolute deflection was 20 μm at 0.4 kHz, (as illustrated in Fig. 2A), all displacements can be assumed linear. In the gerbil and rabbit eardrums, nonlinear displacements have been observed starting from 96 dB SPL [14], [30]. However, the nonlinear component is 100 times weaker than the linear component at those sound levels. Therefore, we assume linearity in our measurements, which were performed at 100 dB SPL.

We found that pure tone displacement amplitudes and multisine displacement amplitudes corresponded closely. However, disk 1, for example, showed significantly lower normalized displacement at 1 kHz under pure tone stimulation compared to the multisine result (see Fig. 2B). We suspect this deviation stems from a calibration inaccuracy in the pure tone stimulus at that frequency. As all other results showed close agreement, we retained this deviating measurement to illustrate the variability that can occur when performing sequential, single-frequency measurements.

For the eardrum data, two important additional factors need to be considered when comparing stimuli.

Firstly, biological tissue is prone to dehydration, which stiffens the response under external load. For the rabbit specifically, dehydration has been shown to significantly influence data interpretability and experimental outcome [22], [31]. To limit the effect of dehydration, the eardrum was imaged from the ear

canal side, keeping the middle ear as intact as possible. Data collection took 30 minutes total per sample. Similar data were collected at the start and end of each sample, and found no noticeable change in displacement amplitudes. In contrast [22], [31] observed clear signs of dehydration in minutes when measuring the eardrum from within the middle ear cavity (middle ear cavity walls fully removed). Therefore, differences between our pure tone and multisine results can be partially attributed to these postmortem effects. Comparing our data to the literature is difficult due to the limited data available. We found a resonance frequency for the Rabbit at 1.4 kHz, while [30] reported a resonance of the rabbit eardrum at 1.8 kHz. However, the referenced work used opened middle ear cavities, so the difference in resonance frequency could be the result of dehydration-induced stiffening.

A second factor affecting data interpretability is related to image segmentation [13], [32]. For the disks, the boundary terminated at the metal holder and was easily discerned in the imaging volume. The rubber itself had a strong signal contrast. Therefore, segmentations took less than 60 minutes for all data per disk. The translucent eardrums, in contrast, had much lower OCT intensities. Segmentations took 30 minutes per stimulus and required much more user judgment to separate the eardrum from background. Each dataset was segmented individually, i.e., the masks for the multisines were not reused for the pure tone datasets. Additionally, the location of the umbo (center of the eardrum) needs to be selected for each dataset to create Fig. 3. We slightly adjusted the Otsu thresholds for creating the eardrum segmentation masks and did not observe significant differences in our results. Similarly, we changed the selected location of the eardrum's center slightly and did not find any change in our results. Segmentation time and artefacts are, therefore, another important consideration. Since the multisine volume only needs to be segmented once, this again minimizes errors due to changes in-between segmentations.

Regarding the displacement surfaces shown in Fig. 4, the comparisons of Figs. 2B and 3B remain consistent. However, to match the visual intensity (color scaling) between the pure tone (top row) and multisine data (bottom row), it was often necessary to lower the upper limit of the multisine displacement scale. For instance, in Fig. 4A, the upper limit is set to 4000 nm/Pa at 600 Hz, whereas the corresponding multisine data in Fig. 4E uses an upper limit of 3500 nm/Pa. This adjustment is consistent with the data shown in Fig. 2B at 600 Hz, where the displacement amplitudes agree as expected. Considering the error margins computed for Figs. 2B and 3B, the differences in color scaling can be attributed to the greater uncertainty in the multisine measurements. Therefore, surface vibration visualizations using multisine excitation yield comparable insights into the modal response, provided that the associated uncertainty, propagated according to Eq. (6), is also included for reference.

In clinical research, hand-held OCT vibrometry systems are being developed to detect middle ear pathologies [5], [17]. A key requirement is rapid data acquisition to minimize motion artifacts, while maintaining high spatial resolution to support detailed diagnostic assessment. Increasing evidence suggests that different middle ear pathologies manifest distinct responses

at specific acoustic frequencies [6], [7], [8], [9]. Although pure tone stimulation remains the clinical gold standard [9], multisine excitation presents a promising alternative by enabling accurate broadband assessment. A potential future strategy could involve using multisine stimulation for initial exploratory screening, followed by targeted pure-tone stimulation at diagnostically relevant frequencies. This combined approach may enhance the detection of pathological signatures and support more informed surgical decision-making.

V. CONCLUSION

We presented the first direct experimental comparison of pure tone and multisine acoustic stimulation in OCT vibrometry, focusing on middle ear mechanics. Using a custom-built system, we measured vibrational responses in both rubber membranes and rabbit eardrums. The multisine curves of the rubber membranes directly showed that their resonance frequencies differed, indicating a difference in stiffness. The rabbit eardrums, on the contrary, had identical resonance frequencies and showed similar modal responses. Pure tones provided precise, high-SNR identification of vibrational modes, while multisine excitation enabled rapid, broadband characterization with comparable spatial patterns, despite slightly higher uncertainty.

Our results demonstrate that multisine stimulation significantly mitigates common experimental limitations such as tissue dehydration and motion artifacts. Moreover, postprocessing of multisine data is much more efficient since only one dataset needs to be segmented. These findings highlight a key trade-off between measurement precision and efficiency and support the adoption of a hybrid strategy in clinical OCT vibrometry: using multisines for fast, exploratory assessment, followed by targeted pure tone measurements for detailed diagnostic evaluation. This approach could accelerate the clinical translation of OCT vibrometry, enabling faster, more informative, and non-invasive diagnosis of middle ear pathologies.

APPENDIX

Disk 2: pure tone versus multisine stimulation

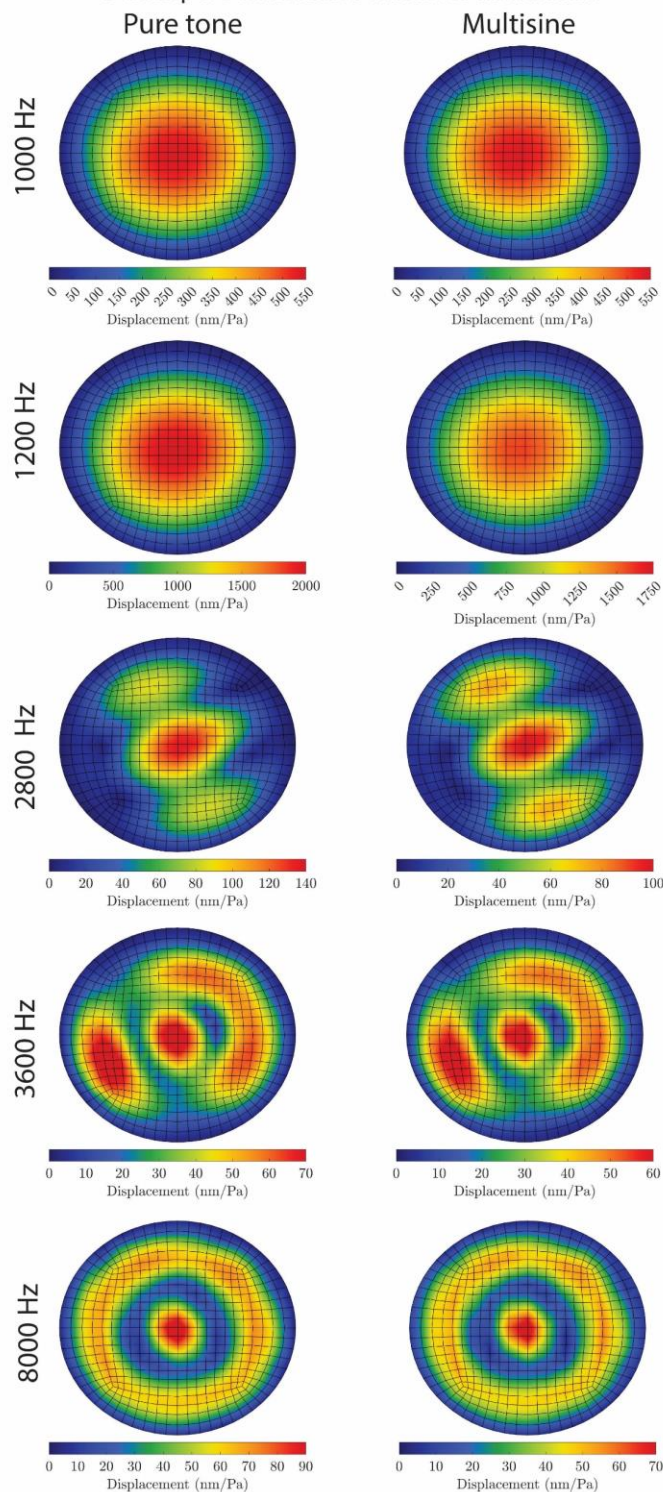


Fig. A1. Comparison of pure tone displacement amplitudes (first column), with the displacement amplitudes of the multisine stimulation (second column). The multisine stimulation results in the same modes shapes as when using pure tones. The uncertainty on the displacement amplitude of the multisines, however, is larger, see Fig. 1.

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