In-plane and out-of-plane motions of the human tympanic membrane

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Computer-controlled digital holographic techniques are developed and used to measure shape and four-dimensional nano-scale displacements of the surface of the tympanic membrane (TM) in cadaveric human ears in response to tonal sounds. The combination of these measurements (shape and sound-induced motions) allows the calculation of the out-of-plane (perpendicular to the surface) and in-plane (tangential) motion components at over 1 000 000 points on the TM surface with a high-degree of accuracy and sensitivity. A general conclusion is that the in-plane motion components are 10–20 dB smaller than the out-of-plane motions. These conditions are most often compromised with higher-frequency sound stimuli where the overall displacements are smaller, or the spatial density of holographic fringes is higher, both of which increase the uncertainty of the measurements. The results are consistent with the TM acting as a Kirchhoff–Love’s thin shell dominated by out-of-plane motion with little in-plane motion, at least with stimulus frequencies up to 8 kHz. © 2016 Acoustical Society of America. [<http://dx.doi.org/10.1121/1.4935386>]

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I. INTRODUCTION

It is recognized that sound-induced motions of the tympanic membrane (TM) are the first step in the transduction of airborne sound energy to the mechanical energy associated with motion of the sensory organs within the inner ear; however, our knowledge of the workings of the TM is rather superficial. While there are multiple model descriptions of TM structure and function, progressing from simple piston models (Shaw and Stinson, 1983), through curved-membrane catenary-dependent models (Goll and Dalhoff, 2011), to complex three-dimensional (3D) finite element models (e.g., Funnell *et al.*, 1987; Williams and Lesser, 1990; Blayney *et al.*, 1997; Gan *et al.*, 2002; Koike *et al.*, 2002; Fay *et al.*, 2005), there is no complete description of how the surface of the TM moves in response to sound to test these models. The most complete descriptions of sound-induced TM motion come from recent stroboscopic holography measurements that quantify the sound-induced displacement at over

500 000 points on the TM surface, but only along a single measurement direction (Cheng *et al.*, 2010; Cheng *et al.*, 2013; Cheng *et al.*, 2015). The detailed TM motion data from such one-dimensional (1D) holographic measurements have been used to better characterize mechanical parameters of the TM, such as damping varying with frequency (De Greef *et al.*, 2014). While some 3D motion measurements describing the displacement of selected regions of the TM exist (Jackson *et al.*, 2012), there has not been a report of the 3D motion of the entire TM surface.

An approximation of such motions has been described from a combination of nano-scale TM displacement measurements with micro-scale measurements of the shape of the external surface of the TM, both made on the TM surface in a cadaveric chinchilla preparation (Rosowski *et al.*, 2013). The technique employed the Kirchhoff–Love approximation of thin shell behavior (which suggests that small motions—much smaller than the thickness of the shell—in shells of the appropriate thickness and shape occur in the direction “normal” to the local surface of the shell) to estimate the motion normal to the surface at each measurement point. This procedure “corrected” the 1D motions measured along the holographic camera axis for significant variations in the direction normal to the curved surface of the TM

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(Rosowski *et al.*, 2013). The computed motions normal to the TM surface showed more spatially uniform patterns in TM motion magnitude than were observed in the raw 1D measurements when the TM ring was not orthogonal to the direction of the sensitivity of the holographic system (Khaleghi *et al.*, 2013; Rosowski *et al.*, 2013).

In the present paper, we expand on our description of TM motion by directly measuring the 3D motion as well as the shape. This methodological advancement is described in the methods section (Sec. II). The combination of 3D motion measurements and shape allow us to not only describe the motions normal to the surface, but now we also quantify motions that are tangential to the local TM surface, the so-called in-plane motions. Quantification of these motions allows us to test the applicability of the Kirchhoff–Love thin shell approximation and also investigate suggestions that in-plane motions are involved in the transformation of acoustic energy into the mechanical energy associated with the motion of the malleus and ossicles (Goll and Dalhoff, 2011; Jackson *et al.*, 2012; Decraemer *et al.*, 2014). A description of our methods and some preliminary results have been published previously (Khaleghi *et al.*, 2015).

II. MATERIAL AND METHODS

A. Preparation and the use of human temporal bones

All of the measurements of TM motion we report here are made in three de-identified normal human temporal bones from donors of age 49 (TM1, male), 77 (TM2, female), and 46 (TM3, female) years. The bones were either fresh or previously frozen at the time of preparation. The preparation of the temporal bones has been described previously (Cheng *et al.*, 2010; Cheng *et al.*, 2013; Cheng *et al.*, 2015) and included (a) removal of the cartilaginous and most of the bony ear canal to expose over 90% of the lateral TM surface, (b) drilling out the mastoid and opening the facial recess to inspect the stapes and round window, and (c) opening the epitympanic space to view the malleus and incus head. After preparation, the bones were lightly fixed in Thiel solution (Thiel, 2002) for more than two weeks; this degree of fixation has been demonstrated to have only small effects on sound-induced stapes and umbo velocities (Stieger *et al.*, 2012), and has been demonstrated to have little effect on the patterns of TM motion in treated human temporal bones (personal observation). The lateral surface of the TM was then painted with a thin coat of zinc oxide (ZnO) suspended in distilled water at a concentration of 60 mg/cc to increase the light reflected from the surface. Such painting has been demonstrated to have little effect on the measured motion (Rosowski *et al.*, 2009; Cheng *et al.*, 2013).

The temporal bones were then mounted on a 3D positioner placed on a vibration-isolated table, which also supported multiple lasers and optical devices needed for the holographic measurements (Hernández-Montes *et al.*, 2009; Flores-Moreno *et al.*, 2011). The bones were positioned such that the planes of their tympanic rings were parallel to the holographic recording CCD camera, and the TM image was centered in the camera plane. The sound stimulus from a speaker mounted on the table was conducted to the ear via a

flexible tube, which terminated several centimeters in front of the lateral surface of the TM. A pre-calibrated probe-tube microphone positioned near the intersection of the TM and its ring was used to record stimulus sound pressure. The stimuli were all continuous tones (Cheng *et al.*, 2010; Cheng *et al.*, 2013).

B. Digital holographic interferometry

1. Holographic interferometry determines the change in the optical path length

Digital holographic interferometry uses changes in the accumulated optical phase between an object and a recording camera plane to quantify the shape or motion of the object (Hernández-Montes *et al.*, 2009; Rosowski *et al.*, 2013; Khaleghi *et al.*, 2015). The differences in optical phase can arise due to motion of the object (Cheng *et al.*, 2010), or a change in the wavelength of the laser illumination of a stationary object (Khaleghi *et al.*, 2013).

a. Measurements of shape using dual-wavelength holographic contouring. Our technique for measuring the shape of the TM has been described in previous articles (Khaleghi *et al.*, 2013; Rosowski *et al.*, 2013). Briefly, the shape is estimated from correlation fringe pattern produced by combining a holographic image gathered using a laser with wavelength λ_1 , and a second image gathered using a different laser wavelength λ_2 . The result is a two-dimensional (2D) interference pattern with fringes that code differences in the optical phase captured on the plane of the recording camera, $\Delta\phi(x, y)$, produced by the two different wavelengths as they propagate along a fixed optical path length (OPL) that includes the distance light travels from the illumination point, to the reflecting surface, and on to the recording camera, i.e.,

$$\Delta\phi(x, y) = \frac{2\pi}{\Lambda} \text{OPL}(x, y), \quad (1)$$

where Λ is the synthetic wavelength defined by

$$\Lambda = \frac{\lambda_1 \lambda_2}{|\lambda_1 - \lambda_2|}. \quad (2)$$

In practice both λ_1 and λ_2 were near 780 nm and differed by ≈ 0.5 nm, such that $\Lambda \approx 1.2$ mm. Since our digital holographic techniques are able to distinguish optical phase difference equivalent to $\sim 1/50$ of a wavelength, the functional sensitivity of our shape measurements is ~ 24 μm .

b. Stroboscopic measurements of motion. Our techniques for determining the sound-induced motion at hundreds of thousands of points on the surface of the TM have been described in detail elsewhere (Hernández-Montes *et al.*, 2009; Cheng *et al.*, 2010; Cheng *et al.*, 2013; Khaleghi *et al.*, 2015). As in the shape determinations, displacement measurements use holographic interference patterns to describe changes in the optical phase; however, the phase changes occur due to change in OPL that results from a

change in the position of the object between two time instants, while the wavelength of illumination λ is fixed. In order to “freeze” the motion of an object vibrating in response to a 0.1–20 kHz acoustic stimulus, we use a high-speed acousto-optic modulator to strobe the illuminating laser such that it is only illuminating the optical path for a brief instant of time during each cycle of acoustic stimulation (Hernández-Montes *et al.*, 2009; Cheng *et al.*, 2010; Khaleghi *et al.*, 2015). In practice, the duration of the illumination is 2%–5% of the period of the acoustic stimulus. The laser “strobe” is triggered at a specific phase of the acoustic stimulus, and for each stimulus frequency eight holographic recordings are performed each at a regularly spaced phase of the acoustic stimulus ($0, \pi/4, \pi/2, \dots, 7\pi/4$). From these recordings we reconstruct the cyclic motion at each point on the surface of the TM. We then use Fourier transforms to describe the magnitude and phase of the motion relative to the magnitude and phase of the acoustic stimulus.

2. Definition of holographic coordinate system and sensitivity vectors

An important part of this paper is our methods for quantifying motion and shape in three dimensions, and fundamental to that discussion is the definition of the directionality of our measurement system, which is summarized in Fig. 1. The nearly planar tympanic ring that supports the outer edge of the TM is positioned in the “object plane,” parallel to the imaging “camera plane” of the digital camera. Both of these planes are orthogonal to the z axis, while the x and y axes fall within the object and camera planes.

While Fig. 1 describes the physical orientation of the camera and the TM, part of our methods includes altering the sensitivity of the holograms recorded by the camera plane to define motions along different directions. Fundamentally, each of our holographic measurements is most sensitive to motion in a single direction and is insensitive to motions orthogonal to that direction. In our case, the direction of sensitivity is altered by varying the direction of the “illumination path” while maintaining a constant “observation path” (Fig. 2). To understand the alteration in directional sensitivity, we need to define the “unit illumination direction vector,” $\bar{K}_I(x, y)$, and the “unit

FIG. 1. (Color online) Definition of the camera based Cartesian coordinates with z pointing toward the camera and x and y defined by the right-hand rule. The camera plane and object plane are parallel.

FIG. 2. A schematic of the determination of the holographic sensitivity vector $\bar{K}_S$ from the unit illumination vector $\bar{K}_I$ and the unit observation direction vector $\bar{K}_O$, where $\bar{K}_S = (2\pi/\lambda)\bar{K}'_S = (2\pi/\lambda)(\bar{K}_O - \bar{K}_I)$. Variations in the direction of $\bar{K}_I$ cause variations in the direction and magnitude of $\bar{K}_S$.

observation direction vector,” $\bar{K}_O(x, y)$ at each point of the recorded hologram (Fig. 2). While $\bar{K}_I$ and $\bar{K}_O$ are dimensionless, the “sensitivity” vector, $\bar{K}_S(x, y)$, is defined by the product of the wave number of the laser light (units of radian-meter^{-1}) and the difference of illumination and observation vectors, where $\bar{K}_S$ is equal to $(2\pi/\lambda)(\bar{K}_O - \bar{K}_I)$, and describes the directional sensitivity of the displacement patterns recorded at each point on the hologram. In other words, holographic interferometry measures motions in the direction of the sensitivity vector of the system with sensitivity proportional to the magnitude of $\bar{K}_S(x, y)$ and, thus, motion components perpendicular to the sensitivity vector are not captured.

3. Transformation of four independent measurements to compute motion along three coordinates

In order to determine all three Cartesian components of displacement vectors, at least three independent equations, obtained by varying the sensitivity vector, $\bar{K}_S$, are needed (Fig. 3). To reduce errors, 2D optical phase change maps were obtained from four sensitivity vectors and the 3D displacement vector at each point on the TM surface were calculated by the least-squares method (Pryputniewicz and Bowley, 1978). At each x, y point on the TM surface, the displacement vector can be obtained by

$$\{d\} = [[S]^T [S]]^{-1} \{[S]^T \{\Omega\}\}, \quad (3)$$

where $\{d\}$ is the 3×1 column vector describing the x , y , and z components of displacement, $[S]$ is the 4×3 sensitivity matrix describing the x , y , and z components of the four sensitivity vectors $\bar{K}_S$, and $\{\Omega\}$ is the 4×1 column vector describing the holographically determined fringe locus functions that describe the motion-induced difference in optical phase in each of the four measurements. The details are provided in Appendix A.

FIG. 3. (Color online) Schematic of the calculation of the magnitude and phase of the Cartesian coordinate representation of the 3D displacements of the TM surface. (1) At all points on the TM surface; (2) we define $\{\Omega\}$ the fringe locus function that describes the optical phase difference due to displacement at eight stimulus phases for all four sensitivity directions; (3) $\{\Omega\}$ and the sensitivity matrix are used to compute $\{d\}$, which describes the three Cartesian components of the displacement at each point, and at each phase of the stimulus; (4) the phasic motion of each Cartesian component is reconstructed; and (5) Fourier transformation defines the magnitude and phase of each of the three Cartesian motion components.

The four illumination directions were chosen to produce linearly independent sensitivity vectors. Due to the inverted conical shape of the TM and the TM's position recessed within the remnant of the boney ear canal, most illumination directions produced noticeable shadows on the TM surface. The illumination paths were chosen to reduce such shadows as much as possible and maintain adequate linear-independence (Khaleghi *et al.*, 2015).

C. Quantification of the magnitude and phase of the 3D motions

The displacement vector $\{d\}$ containing the three Cartesian components of 3D motion was calculated at each of the i points on the TM surface for each of the eight stimulus phases. As shown in Fig. 3, fringe locus functions, Ω_i , corresponding to the sound-induced difference in optical phase at each point on the TM along every sensitivity direction, are stroboscopically measured at eight different instances of the acoustic waveform (Cheng *et al.*, 2010). Using Eq. (3), 3D sound-induced motions of the TM are calculated at each of the eight stimulus phases, and used to define the variation in the component motion in each of the Cartesian directions as a function of stimulus phase. The Fourier magnitudes (d_x , d_y , d_z) and phase-angles (ϕ_x , ϕ_y , ϕ_z) of the phasic displacements of each component were then computed for each point on the TM surface.

D. Use of shape to define in- and out-of-plane motions

According to Kirchhoff–Love thin shell theory, in cases where surface displacements are small relative to shell dimensions, the motion of the TM is well approximated by out-of-plane motions along the direction normal to the TM surface and, consequently, in-plane components of displacement tangent to the surface of the TM are negligible (Kraus, 1967; Saada, 2009). The theory also suggests that knowledge of the unit normal vector η calculated from the shape of the TM at each point on the TM surface (Fig. 4), and a measure of motion in one direction, can be used to compute the

motion normal to the membrane surface d_η . We test the accuracy of the theory, which assumes the in-plane motion is zero, with direct measurement of the in-plane motions and comparisons of the magnitude of in- and out-of-plane motion magnitudes.

Since we also know the shape of the TM we can use the measured x , y , and z displacements at each point on the TM to define motions that are normal to the local TM surface, η (displacements out of the local plane), and motions that are tangential to the local surface (displacements within the local plane, α , β). A numerical rotation matrix $[\mathbb{R}]$ (described in Appendix B) is used to rotate the original Euclidean coordinates of the measuring system (x , y , z) at each point on the TM surface, to a new coordinate system (α , β , η) with unit vectors tangent and normal to the TM surface, so that at each point on the surface of the TM, the new displacement vector $\{d_{\text{Rot}}\}$ can be obtained with

$$\{d_{\text{Rot}}\} = [\mathbb{R}] \times \{d\}. \quad (4)$$

Comparisons of the magnitude of d_η with d_x and d_y describe the relative magnitude of the out-of- and in-plane motions and test whether the TM acts as a thin shell (e.g., Rosowski *et al.*, 2013). It should be noted that the magnitude of the

FIG. 4. (Color online) A 2D view of the 3D shape of a human TM. The illustrated x - z plane is orthogonal to the x - y plane of the camera, which is roughly aligned with the plane of the tympanic ring. Increasing x corresponds to inferior to superior. Increasing y is anterior to posterior. Increasing z is medial to lateral. The umbo is the apex of the conical TM. The inset shows the transformation to the local coordinate system at a point on the TM surface, where $\eta(x, y)$ is normal (out-of-plane) to the membrane surface.

FIG. 5. (Color online) Measured 3D shape of the lateral surfaces of three human TM samples: (a) shape of TM1, (b) shape of TM2, (c) shape of TM3. The black outlines show the borders of the manubrium of the malleus on the medial surface of the TM; (d) cross sections along the manubrium (bounding the superior part of the membrane) of the shapes of all three TM samples; (e) cross sections of the shapes of the three samples along a line through the umbo, but perpendicular to the manubrium; and (f) estimating the individual radii of curvature of the TM by fitting circles to the semi-cross-sections defined by tracing the TM surface between the umbo and the TM rim. In the illustration, the posterior semi-cross-section is fit by a circle of radius R_1 , and the anterior section is fit by R_2 . Also illustrated is the definition of the cone angle γ estimated from this posterior-anterior (P-A) view. The radii of curvature and cone angles are tabulated in Table I.

rotated displacement vector $|d_{\text{Rot}}|$ is equal to the magnitude of the original displacement vector $|d|$, i.e.,

$$|d| = \sqrt{d_x^2 + d_y^2 + d_z^2} = |d_{\text{Rot}}| = \sqrt{d_\alpha^2 + d_\beta^2 + d_\eta^2}. \quad (5)$$

III. RESULTS

A. Measurements of human TM shape

2D projections of the shape of the three human TM are illustrated in the top row of Fig. 5. In each illustration, the umbo is used to define the x , y , z origin, and the manubrium is positioned along the x axis. In each specimen, the TM appears as a blunted cone with a depth of about 2 mm and a radius of about 4 mm. The umbo is the most medial point on the TM (z value of 0) and the z -coordinates of all points on the TM surface are ≥ 0 . Figures 5(d) and 5(e) compare the z -coordinate of different points along two diameters on the TM surfaces: one diameter that includes the manubrium of the malleus and a second perpendicular to the first that also includes the umbo. To evaluate the radii of curvature, the z -coordinates of the shape along lines normal to the manubrium were considered. The radius of curvature of the toroid section of the TM (as per Fay *et al.*, 2005) was calculated with a least-square fit of a circle equation of the outermost part of the TM. As shown in Fig. 5(f), circles are fit to the two sides of the TM [left (R_1) and right (R_2)]. The computed radii of curvature are listed in Table I.

Also included in Table I is a measure of the angle of the cone of the TM estimated from the same diameters, where the angle γ is fit to the steeply sloping sides of the TM, as schematized in Fig. 5(f). Table I lists this angle for both the superior-inferior (S-I) and posterior-anterior (P-A) diameters.

These angles vary between 110 and 133 degrees in our three specimens.

B. Magnitude and phase of motion along different sensitivity vectors

Figure 6 illustrates the measured displacements along four sensitivity vectors in one of the bones (TM1) at 4.48 kHz. The magnitudes and phases at the different points on the TM surface measured along each vector have all been normalized by the complex motion of the umbo obtained from the first sensitivity vector. The displacement magnitude and phases are arranged in complex patterns on the TM surface with on the order of ten local displacement maxima and a similar number of distinct islands of different phase. About half of the membrane moves with a magnitude larger than the umbo displacement. More than half of the membrane moves with a phase that is within ± 0.2 cycles of the umbo, and a large part of the remaining area is nearly moving out of phase with the umbo. Similar patterns have been observed with this frequency of stimulation in other specimens (Rosowski *et al.*, 2009; Cheng *et al.*, 2013; Cheng *et al.*, 2015). The magnitudes and phases of the motions of the surface are similar for the four different sensitivity vectors, though there are small differences in the

TABLE I. Estimation of the radii of curvature and cone angles for three human TM samples.

Radii of curvature and cone angles		TM1	TM2	TM3
R_1 (mm)	S-I	5.84	4.81	3.78
	P-A	4.93	4.96	5.21
R_2 (mm)	S-I	3.41	4.65	4.94
	P-A	4.73	3.95	4.52
Cone angle γ (degrees)	S-I	128	109	127
	P-A	110	105	133

FIG. 6. (Color online) Measurements of the magnitudes and phases of the displacements measured along four different sensitivity vectors ($\bar{K}_1, \dots, \bar{K}_4$) in bone TM1 normalized by the complex motion of the umbo obtained from $\bar{K}_1$. The magnitudes scaled as dB re umbo displacement in $\bar{K}_1$ are in the top row. The phase angles relative to the phase of the umbo in $\bar{K}_1$ are in the bottom row. The stimulus was a 4.48 kHz tone of 106 dB sound pressure level (SPL).

relative magnitudes of the peaks and in the phase of motion. The similarity in displacement across the four sensitivity vectors is consistent with the relatively small differences in illumination directions (span of ± 35 degrees) that were limited by the need to minimize shadowing on the TM from each direction (see Sec. II B 3). Similar results were obtained in the other two bones.

C. Four-dimensional (4D) Measurements: Motion as a function of space (x, y, z) and stimulus phase (θ)

As described in Fig. 3, the 3D position of the TM at each stroboscopic phase (relative to stimulus phase $\theta = 0$) is calculated from the data obtained from all four sensitivity vectors. The three leftmost columns of Fig. 7 show the calculated three Cartesian components of surface motion at six representative acoustic stimulus phases ($\theta = 45^\circ, 90^\circ, 135^\circ, 180^\circ, 225^\circ$, and 270°). The rightmost column shows the computed magnitude of the displacement [Eq. (5)] at each stimulus phase and maps only positive values. While parts of the TM surface move in (negative displacements) and others out (positive displacements), the absolute value of all three components and their combined magnitude increases while θ varies from 45° to 135° , changes little between 135° and 225° , and falls between 225° and 270° of stimulus phase. Based on the progression of surface displacement magnitude with stimulus phase, and considering the overall TM motion, the stimulus phases of $\theta = 0$ and $\theta = 180$ were approximately aligned with instances of minimum and maximum, respectively, in the displacement waveforms on much of the TM surface.

D. Estimates of TM motion in the camera-based Cartesian coordinate system

The displacement components at each point on the TM surface gathered from the four illumination directions

(Fig. 6) were used with Eq. (3) to calculate 3D motions at each stroboscopic phase (Fig. 7) and, then, Fourier transforms were computed of the phasic displacement along each of the three Cartesian axes to compute the magnitudes and phases of motion in the 3D camera-based coordinate system (Fig. 8), where d_x and d_y describe displacements in the measurement plane and d_z describes motions toward and away from the camera. A general result from this calculation is that the x and y components of motion at many locations on the TM surface are 10 dB smaller in magnitude than the z component, though there are regions and frequencies where d_x and d_y are within 5 dB of the magnitude of d_z .

Comparisons of the phase angles of the three components are complicated by the small magnitude of d_x and d_y at many locations on the TM surface. These small displacements approach the noise floor of our measurement system and lead to inaccuracies in the phase estimates that cause the speckling visible in the phase maps in regions of low displacement magnitude. Regardless of the noise-associated speckling, it is clear that the phase of motion in the x and y directions can differ from the phase in the z direction. For example, φ_x , the phase angle of d_x , is generally about 0.5 period out of phase with φ_z , thus, based on our conventions, positive motion along the x axis coincides with negative motion along the z axis.

E. Computed normal and in-TM plane motions

Our methods provide measurements of TM 3D shape and 3D motion that we combine [using Eqs. (3) and (4)] to compute the displacement components that are normal to the TM surface, d_n (out-of-TM plane), and the two displacement components describing the motion in the plane orthogonal to the local normal vector, d_α and d_β (the two components in the local plane of the TM). Figure 9

FIG. 7. (Color online) 4D (x , y , z , and θ) sound-induced motion measurements in TM1 at one single frequency of 4.48 kHz at 106 dB SPL at six instances of θ (the stimulus phase). The stimulus phase goes through a full cycle from the top to the bottom panels. The three left-hand columns are maps of the relative displacement between the stimulus phase and phase zero, and can contain + or – displacement values. The right-hand column shows the magnitude of the displacement [Eq. (5)] at each location on the TM at the different phases and only displays positive values.

FIG. 8. (Color online) The magnitudes and phases of the three camera-based components of the TM displacement in x , y , and z (d_x , d_y , d_z) in TM1. The magnitudes are scaled in dB relative to the displacement of the umbo in the z direction. The phases are scaled in periods relative to the phase of umbo motion in the z direction.

illustrates maps of the magnitude and phase of these three components for TM1. Note that in regions where the magnitude of the motion normal to the TM surface is clearly measurable (≥ 0 dB), the magnitudes of the in-plane motion components $|d_x|$ and $|d_y|$ are generally 10–20 dB smaller than the magnitude of the motions normal to the TM surface $|d_z|$. This point will be re-addressed after we look at the computations of d_z for all three of the temporal bones measured in our series.

Figure 10 illustrates maps of the magnitude of the computed d_z for all three bones measured at similar frequencies. There are many similarities and differences in the set of three results. Similarities include (a) The progression from a few displacement maxima with stimulation near 1 kHz to many local maxima with stimulation near 8 kHz; (b) the arrangement of the multiple maxima around the umbo and

manubrium observed with 4–9 kHz stimulation, and (c) the observation that the magnitude of motion of the umbo and manubrium in the direction normal to the local TM surface is at least 10 dB smaller than the maxima displacements on the TM surface. All of these features have been observed in our previous reports of measurements of z -component motion (Cheng *et al.*, 2010; Cheng *et al.*, 2013; Cheng *et al.*, 2015).

Figure 10 also points out differences in how the TMs of individual temporal bones respond to sound. This is where we see differences in the shape and location of maxima as well as differences in the rate in which the patterns increase in complexity with frequency (Rosowski *et al.*, 2009).

We next address the relative magnitudes of the normal, d_z , and in-plane displacements, d_x and d_y , in the three bones. Figure 11 illustrates the point-by-point ratio of the in- and

FIG. 9. (Color online) The in-plane (d_x and d_y) and out-of-plane (d_z) motion components of TM1 normalized by the out-of-plane motion of the umbo at each frequency. The color bars are in logarithmic magnitude scale, and phases are in cycles.

FIG. 10. (Color online) The magnitude of the motion normal to the TM surface $|d_{\eta}|$ normalized by the sound pressure at the TM at three frequencies in the three temporal bones in our study. The displacement magnitudes are scaled in terms of dB re 100 nm of displacement per Pascal of sound pressure stimulus.

out-of-plane displacement magnitudes, where the in-plane magnitude is defined by the mean of the magnitudes of d_{α} and d_{β} . These ratio maps indicate that over much of the TM surface with stimulus frequencies < 2 kHz, the in-plane motion is much smaller in magnitude (< -10 dB) than the motion normal to the surface (most of the points on the surfaces of the TMs are coded from -30 to -12 dB). The comparison is more complicated at higher frequencies where the regions coded from 0 to 6 dB increase in prominence as frequency increases. Careful comparison of Figs. 10 and 11 point out that regions in Fig. 11, where the displacement ratios are above 0 dB (i.e., in-plane displacement magnitudes $>$ out-of-plane) correspond to regions in Fig. 10 where the motion normal to the surface is of low magnitude. Indeed, many of the regions in Fig. 11 where the ratio is near or

> 0 dB are attributable to regions where $|d_{\eta}|$ is near the measurement noise floor. Therefore, we specified a threshold of 10 nm for the $|d_{\eta}|$, and the value of every single pixel with magnitude smaller than 10 nm are removed from this analysis and their color set to white. Furthermore, since our techniques reconstruct the phasic motion in each of the component directions, we can test for the stimulus-driven sinusoidal response of each component at each pixel by correlating the measured phasic motion with the sinusoidal motion predicted by the fundamental component of the fast Fourier transform (FFT) fit to the motion at each pixel (Cheng *et al.*, 2010). Pixels of poor correlation (with squared correlation coefficients values < 0.7) are also shown as white in these displays. The numbers under each plot panel in Fig. 11 note the average of the dB values in each panel.

FIG. 11. (Color online) Maps of the ratio of in- to out-of-plane where in-plane is defined by the mean of the magnitudes of the two in-plane motion components. Image pixels where the magnitude of motion of any of the component motions was < 10 nm, or where the correlation between the sinusoidal stimulus and the motion had a square of the correlation coefficient of < 0.7 are colored white.

The consistent reading near -10 dB say that, on average, the in-plane motion components are $<30\%$ of the magnitude of the out-of-plane motion component.

IV. DISCUSSION

New computer-controlled holographic techniques have been used to quantify 3D spatial displacement components at over a million points on the surface of the TM in response to tonal sound stimulation and (nearly simultaneously and using the same observation angle) determine the 3D shape of the lateral surface of the TM. It should be noted that some other researchers have previously reported measurements of 3D shape of the TM, e.g., Moiré interferometry (Decraemer *et al.*, 1991); however, the advantages of our new holographic techniques are, first, this technique does not require any triangulation between the observation (camera) and illumination (light projector) points, which is typically the case of structured light interferometry and, second, both sound-induced motion and 3D shape of the TM can be quantified with one single measuring system. This combination of measurements allows us to quantify the out-of-plane displacements (the displacement component perpendicular to the local surface) and the in-plane displacements (the displacement components tangential to the local surface) associated with sound-induced TM motion.

A. Description of the multiple directional components of TM surface motion

Our methods use four independent measurements of the sound-induced motion of the TM, with each measurement associated with a different illumination direction and holographic sensitivity vector $\overline{K_S}$ (Fig. 2). Because the variation in the sensitivity vector was limited by the geometry of the TM and its supporting bone, measurements made with the four illumination directions were similar (but not identical) in magnitude and phase (Fig. 6). The known sensitivity vectors and the four measurements were used in a least-squares calculation [Eq. (3), Appendix A] to define the motion of over 1 000 000 points on the TM surface in terms of the Cartesian coordinates (d_x , d_y , and d_z) imposed by the camera plane, with the z direction orthogonal to the plane. Because of the flattened cone shape of the TM, the measurement geometry with the TM ring paralleling the camera plane and the limited variations in the directions of the four sensitivity vectors (<30 degree relative to the z direction), the computed d_z is generally similar in magnitude and phase to the displacements measured with the four different illumination directions (Figs. 6 and 8).

B. Comparison of out-of-plane motion and motion orthogonal to the tympanic ring

Our new measurements allow us to assess the out-of-plane (normal to the local surface) component d_η of TM motions and compare it to our previous measurements of motion orthogonal to the tympanic ring (Cheng *et al.*, 2010; Cheng *et al.*, 2013; Cheng *et al.*, 2015) that generally correspond to our d_z measurements. Direct comparison of

measurements in TM1 at 4.48 kHz show great similarity between d_z (Fig. 8, right-hand column) and the computed d_η (Fig. 9, right-hand column, center rows), though there are differences, particularly in areas midway between the center of the TM and its rim. The location of these differences corresponds to regions where the curvature of the TM is largest, while the regions of similarity are explainable in terms of the near orthogonal orientation of the TM ring (and the center of the TM) with the z axis of the camera-defined coordinate system employed in our methods. In chinchilla, where because of the anatomy of the ear canal it is necessary to place the TM at an angle to the camera plane in order to view the entire TM surface, bigger differences were observed between d_z and d_η (Rosowski *et al.*, 2013).

Further comparisons of the d_η magnitude maps of Fig. 10 with similar maps in the literature show great similarities between the measured normal component of motion and the previously published 1D measurements made along the z axis in our previous reports (Cheng *et al.*, 2010; Cheng *et al.*, 2013; Cheng *et al.*, 2015). The d_η data from this study illustrated in Figs. 9 and 10 all show the relatively low magnitude of motion of the umbo at all stimulus frequencies, and the frequency dependent evolution of displacement patterns that we have previously described in our 1D measurements of TM motion. These include (1) The presence of one or two displacement maxima and generally in phase motion of the entire TM surface near 1 kHz and at lower frequencies, (2) the increase in the number of local displacement maxima with frequency coupled to the introduction of phase variations on the TM surface with stimulus frequencies between 1 and 5 kHz, and (3) the ring-like organization of maxima and iso-phase islands that are circularly arranged around the umbo in the center of the TM at frequencies above 6 kHz.

C. Comparison of in-plane displacements (d_α and d_β) with d_x and d_y

Our methods also provide a measurement of the x and y components of displacement d_x and d_y , as well as the two components of in-plane displacements (d_α and d_β) on the TM (those tangent to the local surface). While d_α and d_β components were measureable, they were generally small, and our estimates of their magnitude and angle were often limited by our ability to resolve these components (Fig. 9). The motion magnitudes in the x and y were generally larger in magnitude than the in-plane components (e.g., Figs. 8 and 9). This is consistent with the cone shape of the TM and a motion that is dominated by displacements normal to the cone's surface. Because of the orientation of the TM cone relative to the x - y plane define by the tympanic ring, motions normal to the surface can have significant x and y motion components, even when the in-plane motions are zero in magnitude. The importance of the TM shape in relating d_α and d_β to d_x and d_y suggests that measurements of the latter two, without knowing shape, are not useful estimates of in-plane TM motions.

D. What is the relative magnitude of in- and out-of-plane motions?

Because of the large variations we observed in the magnitude of d_x and d_y , we performed more direct comparisons of d_x and d_y , and d_z . These are illustrated in Fig. 11 as maps of point-by-point computations of the mean magnitude of d_x and d_y divided by d_z magnitude, where this ratio is scaled in dB. In Fig. 11, 0 dB is assigned to those regions where the in- and out-of-plane displacement magnitudes are equal, pixels with values higher than 0 dB code regions where the in-plane motions are larger than the out-of-plane, and pixels with values smaller than 0 dB correspond to regions where the out-of-plane motions are larger in magnitude. The gestalt from these plots is that out-of-plane motions are generally larger than the in-plane motions. More quantitatively, the ratio of in- to out-of-plane motion varies over the surface of the TM between 6 and -30 dB with an average value near -10 dB. As noted in Sec. III, this comparison is complicated when the motion of the out-of-plane component is small and near the magnitude of the measurement noise floor and many, but not all, of the locations where the computed ratio is 0 dB or above surround locations where the measured displacements are small and our displacement estimates are known to be noisy.

Our estimates of the magnitudes of in-plane motion components were not always small, especially in isolated spatial regions in response to stimuli of frequencies above a few kHz. For example, in Fig. 9, the d_x and d_y data with the 4.48 kHz stimulus show several small punctate regions just to the right of the manubrium, where these “in-plane” motions are larger than the out-of-plane $|d_z|$ motions of the umbo. Small, localized regions near these same locations also show d_x and d_y magnitudes larger than the out-of-plane motion of the umbo with 8 kHz stimulation. The highly localized nature of these regions argues against any functional significance of these puncta where the in-plane motions are large in magnitude.

E. Does the TM act as a thin shell?

The Kirchhoff–Love theory of thin shells describes a system dominated by out-of-plane displacements (displacements normal to the shell mid-surface; Kraus, 1967; Saada, 2009). Our measurements of 3D sound-induced motions of the cadaveric human TM and its 3D shape suggest that out-of-plane motions generally dominate the sound-induced motion of the TM; however, our data do suggest the presence of in-plane motions and, in isolated regions of the TM, the in-plane motions can be larger than the out-of-plane motions.

Thin shell theory generally applies to shells made of homogenous material, in which the resultant displacements are small compared to the thickness of the shell, and the thickness of the shell is <0.05 of the radius of curvature of the shell. Our data and TM preparations are consistent with both of the numerical constraints: The thickness of the human TM varies between 50 and 140 μm (Van der Jeught *et al.*, 2013), and the largest displacements we observe are $<3 \mu\text{m}$, which leads to a displacement to thickness ratio of <0.06 .

Also, the radii of curvature in the three TMs varies between 3.4 and 5.8 mm (Table I) yielding a ratio of max thickness to this radius of <0.045 . However, the TM is not homogenous.

The normal TM is a tri-laminar structure in which the central layer contains several populations of fibers with different orientations (Decraemer and Funnell, 2008), a structure that is not consistent with homogeneity. Furthermore, as ears age, the TM is subject to multiple subclinical alterations in structure, e.g., tympanosclerosis (Merchant *et al.*, 2010). The events can cause scars or mineral deposits in the TM that further impact the heterogeneity of its structure. While each of the three ears used in our study had no overt signs of middle ear disease, none had the clear pristine TM generally observed in children and young adults. Furthermore, non-uniformities in the painting of the TM surface with the ZnO solution are also possible; the TM surface is somewhat hydrophobic and the water-based solution does not always cover the surface equally. Whether paint-induced or natural structural inhomogeneities were associated with the punctate regions of clearly large in-plane displacements is a point of further study.

V. SUMMARY AND CONCLUSION

We have described new holographic methods that can measure the 3D shape and 3D motion of the surface of the TM nearly simultaneously using a fixed geometry of TM specimen and digital camera back plane (Khaleghi *et al.*, 2013; Khaleghi *et al.*, 2015). The combination of shape and 3D motion was used to produce the first accurate measurements that separate the out-of-plane (normal to the surface) and in-plane (tangential to the surface) displacement components. While the latter were shown to be generally small, consistent with thin shell theory, they were difficult to quantify when the membrane motion was small. There were also small regions of the membrane surface with in-plane motions that were larger in magnitude than the measured out-of-plane motions.

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APPENDIX A: 3D MOTION MEASUREMENTS WITH THE METHOD OF MULTIPLE SENSITIVITY VECTORS

As mentioned in Sec. II B 3, 3D motion components of the TMs are measured with the method of multiple sensitivity vectors in holographic interferometry. The difference in optical phase Ω produced by a displacement $\vec{d}$ measured with sensitivity vector $\vec{K}_S$ is the dot product of the sensitivity vector with the object's displacement vector

$$\Omega = \vec{K}_S \cdot \vec{d}, \quad (\text{A1})$$

where the sensitivity vector is the product of the light wave number and the vector difference between the unit observation vector and the unit illumination vector (Fig. 2). In the case of a 3D motion and a sensitivity vector described in three dimensions, Eq. (A1) can be expanded to

$$\begin{aligned}\Omega &= (K_{Sx}, K_{Sy}, K_{Sz}) \cdot (d_x, d_y, d_z) \\ &= K_{Sx}d_x + K_{Sy}d_y + K_{Sz}d_z.\end{aligned}\quad (\text{A2})$$

If we know Ω and $\overline{K_S}$, we have one equation and three unknowns, d_x , d_y , and d_z . In order to solve Eq. (A2), at least three linearly independent sensitivity vectors are required: $\overline{K}_1$, $\overline{K}_2$, and $\overline{K}_3$. In order to minimize experimental errors, we obtain optical phase maps using four different sensitivity vectors to form an overdetermined system of equations of

$$\begin{bmatrix} \Omega_1 \\ \Omega_2 \\ \Omega_3 \\ \Omega_4 \end{bmatrix} = \begin{bmatrix} K_x^1 & K_y^1 & K_z^1 \\ K_x^2 & K_y^2 & K_z^2 \\ K_x^3 & K_y^3 & K_z^3 \\ K_x^4 & K_y^4 & K_z^4 \end{bmatrix} \times \begin{bmatrix} d_x \\ d_y \\ d_z \end{bmatrix}, \quad (\text{A3})$$

which can be simplified into the following equation:

$$\{\Omega\}_{4 \times 1} = [\mathbb{S}]_{4 \times 3} \times \{d\}_{3 \times 1}. \quad (\text{A4})$$

Equation (A4) is solved with the least-squares error minimization method. First, both sides are multiplied by the transpose of the sensitivity matrix, $\mathbb{S}$,

$$[\mathbb{S}]_{3 \times 4}^T \times \{\Omega\}_{4 \times 1} = [\mathbb{S}]_{3 \times 4}^T \times [\mathbb{S}]_{4 \times 3} \times \{d\}_{3 \times 1}. \quad (\text{A5})$$

Thus, the displacement vector $\{d\}$ can be obtained by

$$\{d\}_{3 \times 1} = [[\mathbb{S}]_{3 \times 4}^T \times [\mathbb{S}]_{4 \times 3}]_{3 \times 3}^{-1} \times \{[\mathbb{S}]_{3 \times 4}^T \times \{\Omega\}_{4 \times 1}\}_{3 \times 1}. \quad (\text{A6})$$

An important consideration in the method of multiple sensitivity vectors is that all the sensitivity vectors need to be as linearly independent as possible for the system to provide accurate results. Therefore, the condition number, $\mathbb{C}$, of the square matrix, $[F] = [\mathbb{S}]^T [\mathbb{S}]$, characterizing the geometry of a holographic setup is calculated (Vest, 1979)

$$\mathbb{C}(\mathbb{S}) = \|\mathbb{S}\| \cdot \|\mathbb{S}^{-1}\| = \sqrt{\frac{\lambda_{\max}(F)}{\lambda_{\min}(F)}}, \quad (\text{A7})$$

where $\|\mathbb{S}\|$ is the norm of the matrix $\mathbb{S}$, and $\lambda_{\max}$ and $\lambda_{\min}$ are the maximum and the minimum eigenvalues, respectively, of $[F]$. A condition number close to one indicates a well-conditioned matrix, but this represents a holographic setup with large angles of illumination (Vest, 1979; Osten, 1985). However, because of the particular cone-like geometry of the TM and the presence of the bony structures around it, the maximum possible angles of illumination are limited. Therefore, we arranged our different illumination directions to achieve the largest angles of illumination within the constraints imposed by the geometry of the TM. In our holographic system, a condition number of 4.7 has been achieved, which corresponds to a well-conditioned holographic system (Vest, 1979; Osten, 1985). In addition, the accuracy of the measurements obtained with this holographic system has been previously verified by another documented method (Khaleghi *et al.*, 2015).

APPENDIX B: ROTATION MATRIX USED TO OBTAIN IN- AND OUT-OF-PLANE MOTIONS

The original Cartesian coordinate system x, y, z is mathematically rotated in order to obtain the local in- and out-of-plane displacement components. In the holographic system and based on the definition of the sensitivity vectors (Fig. 2), the observation vector $\overline{Z}$, i.e., a vector perpendicular to the CCD sensor, has unit vector components $\overline{Z}_x$, $\overline{Z}_y$, and $\overline{Z}_z$ with magnitudes equal to $[0, 0, 1]$. From the measured 3D shape of the membrane, we can define the unit normal vector, $\overline{N}$, at every point on the surface of the TM (Khaleghi *et al.*, 2013). Since, both $\overline{N}$ and $\overline{Z}$ are unit vectors, the angle θ between them is calculated as the dot product of the two vectors; and the cross product of these two vectors provides a vector, $\overline{U}$, normal to both of them that, in this case, is tangent to the local plane of the membrane and is considered as the axis of rotation.

The rotation matrix, $\mathbb{R}$, is used to rotate the original displacement vector $d(d_x, d_y, d_z)$, based on the rotation angle θ and the unit vector of the axis of rotation $\overline{U}$ with

$$\mathbb{R} = \begin{bmatrix} \cos \theta + U_1^2(1 - \cos \theta) & U_1 U_2(1 - \cos \theta) - U_3 \sin \theta & U_1 U_3(1 - \cos \theta) + U_2 \sin \theta \\ U_2 U_1(1 - \cos \theta) + U_3 \sin \theta & \cos \theta + U_2^2(1 - \cos \theta) & U_2 U_3(1 - \cos \theta) - U_1 \sin \theta \\ U_3 U_1(1 - \cos \theta) - U_2 \sin \theta & U_3 U_2(1 - \cos \theta) + U_1 \sin \theta & \cos \theta + U_3^2(1 - \cos \theta) \end{bmatrix}, \quad (\text{B1})$$

where $\overline{U}(U_1, U_2, U_3)$ is the unit vector of the axis of rotation of the observation direction (z axis in the original measuring coordinate system) and θ is the angle of rotation. Therefore, as shown in Fig. 12, at each point m, n , the rotated displacement vector d_{Rot} has components tangent

(d_α and d_β) and normal (d_η) to the local TM plane and is calculated with the matrix multiplication of the rotation matrix, $\mathbb{R}$, with the original displacement vector d with

$$d_{\text{Rot}}(m, n) = \mathbb{R} \times d(m, n). \quad (\text{B2})$$

FIG. 12. (Color online) Transformation of the measuring coordinate system to the local coordinate system of the TM by means of a rotation matrix. (Left) The definition of $\vec{N}$, the normal vector, and $\vec{U}$, the axis of rotation between $\vec{N}$ and the observation direction, $\vec{Z}$. $\vec{U}$ is perpendicular to both $\vec{N}$ and $\vec{Z}$ and within the plane of the TM. (Middle) The transformation between the Cartesian coordinates defined by the camera plane (x, y) and observation direction (z) and the out-of-plane (η normal to the local surface) and in-plane (α and β , tangential to the local surface) motion directions.

APPENDIX C: PROCEDURES FOR 3D SHAPE MEASUREMENTS

The four-phase-stepping holographic contouring technique (Furlong and Pryputniewicz, 2000; Khaleghi *et al.*, 2015) is used to quantify the 3D shape of several human TM samples. As shown in Fig. 13(a), the CCD sensor is illuminated with both reference and object beams at wavelength λ_1 . The camera captures four intensity patterns I_1 – I_4 as four consecutive camera frames gathered with accumulating $\pi/2$

phase steps added to the optical path of the reference beam. Then, the laser is tuned to a new wavelength λ_2 and another set of four intensity patterns at the second wavelength are recorded. The optical phases at each of these two states are calculated as shown in Fig. 13(b). Then, the differences between the optical phases of the two states are calculated in order to obtain a fringe pattern corresponding to the shape of the TM, which along with 3D displacement measurements is used to determine the in- and out-of-plane motions. For the purposes of displaying the shape in 3D, we remove any

FIG. 13. (Color online) Algorithms used to calculate 3D shape of the TMs using dual-wavelength holographic contouring: (a) four phase-stepped intensity maps are captured by the CCD sensor and two digital holograms at two different wavelengths λ_1 and λ_2 are recorded, (b) both holograms are reconstructed and optical phases corresponding to each hologram are obtained, (c) the optical phase difference between the two holograms is obtained and a numerical plane subtraction is implemented to remove the carrier fringes related to the geometry of the optical setup, and (d) the optical phases difference is unwrapped and scaled to realize the 3D shape of the TM.

biases introduced by the orientation of the TM and the illumination directions. Such biases introduce regular carrier fringes overlaid on top of the shape-related fringes (Vest, 1979). Therefore, a numerical plane is subtracted to mathematically remove the carrier fringes, and the data are masked and scaled in order to obtain the unbiased 3D shape of the object.

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