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Tympanic membrane contour measurement with two source positions in digital holographic interferometry

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Abstract: The data acquisition from the shape of an object is a must to complete its quantitative displacement measurement analysis. Over the past years whole field of view optical non-invasive testing has been widely used in many areas, from industrial ones to, for instance, biomedical research topics. To measure the surface contour from the tympanic membrane (TM) of ex-vivo cats digital holographic interferometry (DHI) is used in combination with a two-illumination positions method: the shape is directly measured from the phase change between two source positions by means of a digital Fourier transform method. The TM shape data in conjunction with its displacement data renders a complete and accurate description of the TM deformation, a feature that no doubt will serve to better comprehend the hearing process. Acquiring knowledge from the tissue shape indicates a mechanical behavior and, indirectly, an alteration in the physiological structure due to middle ear diseases or damages in the tissue that can deteriorate sound transmission. The TM shape contour was successfully measured by using two source positions within DHI showing that the TM has a conical shape. Its maximum depth was found to be 2 mm, considering the umbo as the reference point with respect to the TM annulus plane, where the setup is arranged in such a manner that it is capable of measuring a height of up to 7 mm.

OCIS codes: (090.0090) Holography; (120.0120) Instrumentation, measurement, and metrology; (170.0170) Medical optics and biotechnology.

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1. Introduction

Shape measurement of an object facilitates the understanding of its working mechanisms in several phenomena in different scientific research areas as well as in technology and industry. For this purpose, different mechanical tests and numerous non-invasive optical methods for shape measurement have been developed [1–7]. Optical methods in particular have undergone a variety of innovations due to the continuous and rapid advances in the hardware used in them, namely electronic and computer-processor devices. In consequence, optical methods such as Moiré, Fringe Projection and Digital Holographic Interferometry (DHI) can acquire more precise object shape data with higher resolution.

DHI is used in combination with two source positions to measure the TM shape. DHI has in the past demonstrated its usefulness in the quantification of displacements and vibration analysis of diverse solid samples, and in the quantification of refractive-index changes in transparent objects, among many other successful applications [8–11]. Furthermore, it is a non-invasive technique that provides full field-of-view measurements of static and dynamic events by means of the analysis of wavefronts recorded at different points in time which provide useful information to determine various important parameters from the object under study, e.g., amplitude and direction displacements or stress and strain values.

Due to its simplicity the two source positions technique is used to measure shapes in a wide range of applications. It consists in moving the object illumination source from one position to another in a typical-simpler optical out of plane setup (see Fig. 1). This is compared to techniques such as fringe projection, moiré interferometry, or low coherence interferometry, just to mention a few, where the setups and hardware employed may be more complicated to implement for this particular application.

The tympanic membrane has been studied with different techniques capable of measuring surface mechanical parameters and displacements [12–16]. However, most of them do not consider the shape in their data analyses. The TM delicate tissue is crucial to the hearing process in the middle ear structure for it vibrates beneath sound pressure. If the TM undergoes changes in its structure and mechanical properties due to ear disease, sound transmission can be deteriorated and loss of conductive hearing may be caused. Several works have been devoted to TM shape measurements by means of optical methods, but the most of them use moiré interferometry and fringe projection with a phase shifting method [17-21].

This paper's main goal rests at measuring the TM shape of an ex-vivo cat by means of two object source illumination positions in combination with digital holographic interferometry. In a typical out of plane setup, the light from the dividing beam splitter is conveyed through an optical fiber that mounted in a connector adaptor is used to launch the object illumination beam. A first digital hologram is recorded with the object beam at illumination point 1, and then the optical fiber is slightly translated (by a few micrometers) to illumination point 2, and this serves to record the second digital hologram. The optimum object illumination direction is usually determined by a trade-off between the maximum setup sensitivity and minimum shadows casted in the reconstructed image. The shape is directly determined from the phase change between those two source positions, where it is calculated from the recorded digital hologram intensities using a digital Fourier transform method. Experiments were first carried out using a metal sphere to verify the procedure, and once proved repeatable it was applied to measure the TM shape which was found to have a conical shape, with a maximum depth of 2 mm, considering the umbo as the reference point with respect to the TM annulus plane.

This above procedure is fast in that it requires only two images and uses the same optical setup to quantify motion displacements, also avoiding the commonly found rigid body motion that might produce inaccuracies during the data acquisition procedure. It may be safely stated that the main contribution of this work is the finding of the TM shape using digital holographic interferometry, since more accurate and reliable information can be given in combination with displacement measurements, thus completing the study of the TM physiological behavior. With these advantageous features, DHI proves to be best suited to study biological samples.

2. Shape determination

The setup used for the measurement of the TM shape is shown in Fig. 1. This system is based on digital holography interferometry. The laser source (532 nm) is divided into two beams, one serves as the reference beam and the other as the object beam. Both are coupled to an optical fiber launcher, allowing the easiness to place the reference/object beams in any position. Only the object beam is mounted in a micrometer translation stage. The backscattered light coming from the illuminated object is collected by a doublet lens of 30 mm focal length that is placed 5 cm away from the camera CCD sensor. The object and reference beam are recombined by means of a beam combiner on the CCD sensor that registers the digital holographic interferograms finally stored and processed in a computer.

The process for recording the holograms with the two source positions is described in references [6,22]. A first digital holographic interferogram is recorded with the object beam at point p_1; then, the optical fiber is slightly moved laterally to point p_2 to record the second digital holographic interferogram. The phase reconstruction is achieved by means of the Fourier transform method which allows us to obtain the wavefront complex amplitude and phase for each hologram.

The surface shape can be decoded by calculating the Fourier transform, followed by a dedicated filter in its spatial frequency domain, and finally calculating its inverse Fourier transform. Briefly in mathematical terms, the latter procedure is described as follows: first the CCD records an intensity given by

$$\begin{aligned} I(x_H, y_H) &= \left|R(x_H, y_H) + U(x_H, y_H)\right|^2 \\ &= \left|R(x_H, y_H)\right|^2 + \left|U(x_H, y_H)\right|^2 \\ &\quad + R(x_H, y_H)U^*(x_H, y_H) + R^*(x_H, y_H)U(x_H, y_H), \end{aligned} \tag{1}$$

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where x_H and y_H are the coordinates on the hologram plane (CCD sensor), and the asterisk means the complex amplitude conjugate, with

$$U(x_H, y_H) = u(x_H, y_H)\exp\left[i\phi_u(x_H, y_H)\right]$$

and

$$R(x_H, y_H) = r(x_H, y_H)\exp\left[i\phi_r(x_H, y_H) - 2\pi i(f_x x_H, f_y y_H)\right],$$

in which, $r(x_H, y_H)$ is the smooth reference wave, and $u(x_H, y_H)$ is an object wave, with $i = \sqrt{-1}$. The intensity becomes

$$\begin{aligned} I(x_H, y_H) = a(x_H, y_H) + c(x_H, y_H)\exp\left[2\pi i\left(f_x x_H + f_y y_H\right)\right] \\ + c^*\left(x_H, y_H\right)\left[-2\pi i\left(f_x x_H + f_y y_H\right)\right], \end{aligned} \tag{2}$$

where

$$a\left(x_H, y_H\right) = u^2\left(x_H, y_H\right) + r^2\left(x_H, y_H\right)$$

and

$$c\left(x_H, y_H\right) = u\left(x_H, y_H\right) r\left(x_H, y_H\right)\exp\left[i\varphi\left(x_H, y_H\right)\right],$$

where $\varphi(x_H, y_H) = \phi_u(x_H, y_H) - \phi_r(x_H, y_H)$ and applying the Fourier transform to Eq. (2) gives

$$TF\left[I\right] = A\left(f_x, f_y\right) + C\left(f_x, f_y\right) + C^*\left(f_x, f_y\right). \tag{3}$$

Here C and C^* indicate a complex-conjugate pair, each containing the object phase information $\varphi\left(x, y\right)$.

In the spatial Fourier domain they are separated from one another due to a carrier introduced by tilting slightly the beam combiner located in front of the CCD sensor. Only one term, C or C^* is chosen and band-pass filtered, with the term $A(f_x, f_y)$ filtered out at this stage. The inverse Fourier transform is applied and is given complex number, $c(x, y)$.

The resultant phase for each of the two illumination positions is given by

$$\varphi(x, y) = \tan^{-1}\left[\frac{\operatorname{Im} c(x, y)}{\operatorname{Re} c(x, y)}\right], \tag{4}$$

where Im and Re denote the imaginary and real parts of $c(x, y)$, respectively.

Finally, the object shape is found by the phase-difference from φ_1 and φ_2; i.e., the TM surface contour height change is given by

$$\begin{aligned} \Delta\varphi(x, y) &= \varphi_2(x, y) - \varphi_1(x, y) \\ &= \tan^{-1}\frac{\operatorname{Re}(c_1(x, y))\operatorname{Im}(c_2(x, y)) - \operatorname{Im}(c_1(x, y))\operatorname{Re} c_2(x, y))}{\operatorname{Im}(c_1(x, y))\operatorname{Im}(c_2(x, y)) + \operatorname{Re}(c_1(x, y))\operatorname{Re} c_2(x, y))}. \end{aligned} \tag{5}$$

$\Delta\varphi(x, y)$ is related with the change in optical path length sensitivity of the setup which is given by the geometrical optics arrangement: it is determined by the illumination and observation directions. In Fig. 1, the illumination direction is denoted with the unitary vector $\hat{k}_1$. The observation direction is denoted with the unitary vector $\hat{k}_0$.

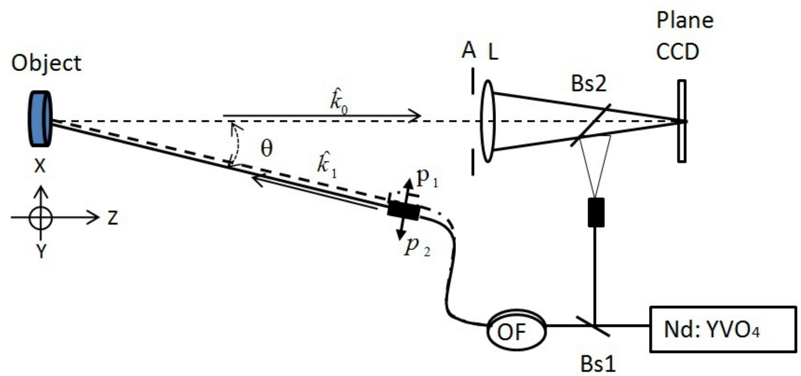

Fig. 1. Experimental optical arrangement: Bs1, beam splitter; Bs2, beam combiner; L, lens; A, aperture; OF, single mode optical fiber; p1, p2, object illumination positions.

The object beam angle θ is measured from the fixed position of object illumination to the axis perpendicular to the geometrical center of the CCD sensor $\hat{k}_0$. The angular displacement $\Delta\theta$ is determined by the source of object illumination that was slightly translated from one point to another p_1–p_2, using a micrometric screw. The measurement of the contour of the sample is obtained by subtracting the phase of the two holograms acquired at two different illumination positions, as described above. Performing some geometrical calculations, that phase-difference is now written in terms of θ and $\Delta\theta$ as

$$\Delta\varphi(x,y) = 2\mathrm{k}\sin\frac{\Delta\theta}{2}x\cos\left(\theta+\frac{\Delta\theta}{2}\right) - 2\mathrm{k}\sin\frac{\Delta\theta}{2}\mathrm{h}(x,y)\sin\left(\theta+\frac{\Delta\theta}{2}\right), \qquad (6)$$

where $\mathrm{k} = 2\pi/\lambda$. Therefore, we can determine the object height-change $\mathrm{h} = (x, y)$ that results from the difference between the reconstructed phases, φ_1 and φ_2, which were recorded before and after a small tilt $\Delta\theta$ introduced in the object illuminating beam. Equation (6) is the same as the one found in Ref. [22], where the object shape is found using phase shifting and a reconstruction algorithm in a rather similar procedure than the one used here.

The following approximation, for small angles, serves to evaluate the setup sensitivity (Δh) for the TM height,

$$\Delta h \approx \frac{\lambda}{\Delta\theta\sin\theta}, \qquad (7)$$

which results in 7mm.

3. Results

As a first test, we measured the shape of a metal sphere of 5 mm radius with an illumination angle θ set at 36° with respect to the CCD sensor axis. The centre of the sphere was positioned at a distance Z = 300 mm from the CCD sensor set to z = 0, i.e., the origin of the coordinate system. The images were recorded with a 1392x1024 pixels CCD camera at 12 bit resolution. The camera acquisition speed is 11 fps, and the interframing time is 5 µs. The total recording time is approximately 180 ms. It is necessary to add the time required for the processing of holograms and the TM contour calculation. The total time to display the TM contour results is about two minutes.

Figure 2a represents the distribution of the wrapped phase, where the object is surrounded by a mask to prevent unwanted noise in the reconstruction. The phase unwrapping image corresponding to a sloped sphere is shown in Fig. 2b. The tilt component must be removed by

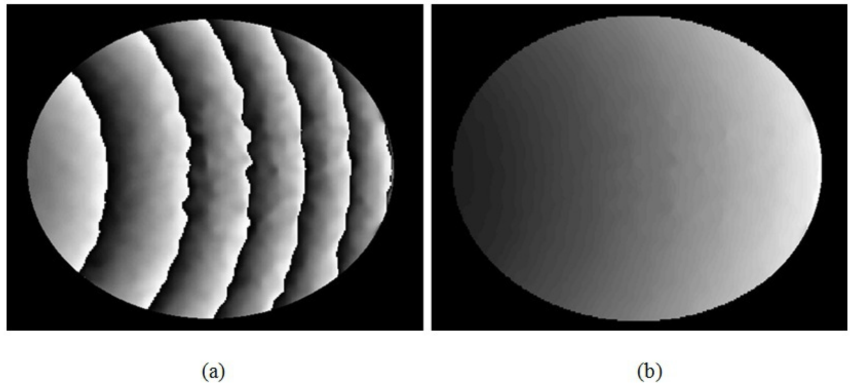

Fig. 2. Experimental surface contour of a metal sphere using a two-illumination positions method: (a) wrapped phase, and (b) unwrapped phase maps with tilt.

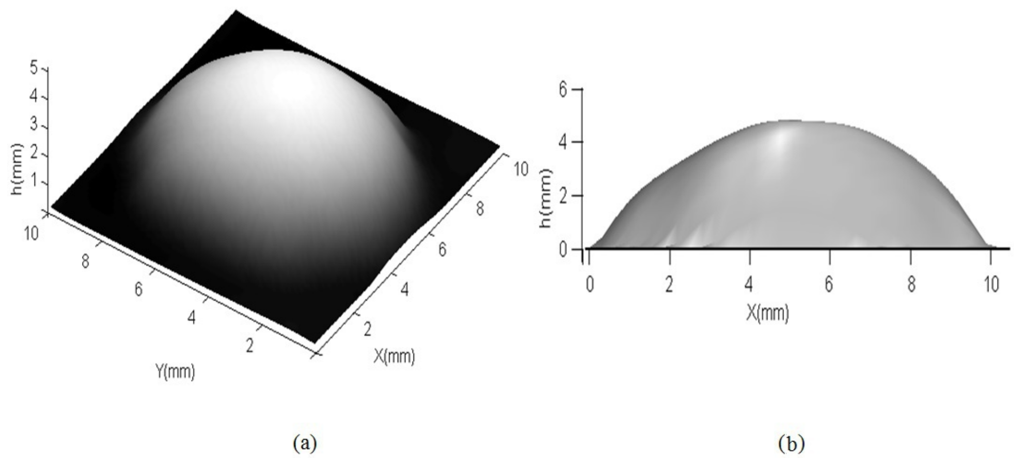

Fig. 3. (a) 3D unwrapped phase map resulting after tilt removing and (b) represent the size view.

the subtraction of the first term in Eq. (6), see Fig. 3. We also calculated the error in the optical phase between two adjacent phase contours resulting in an error value of ~π/12.

The sensitivity of the system is determined by the wavelength and the geometry of the experimental setup such that the angle produced by the source of illumination that was slightly translated from one point to another, can be simply changed to obtain different sensitivity values. This feature makes it possible to set up the tilt angle using a micrometric screw to guide the optical fiber that illuminates the object. In our test, the small angle was fixed at $\Delta\theta = 0.007^{o}$. If is considered that the error introduced by the micrometer screw is half of its smallest division, 12.5 micrometers in the displacement, the error in the angle is $\pm 0.0017°$ that results in a measurement capability ranging from 6.4 mm to 9.7 mm. However, extreme care was taken to make the measurements repeatable to minimize or better make this error negligible.

Reference [23] describes the manner in which the tissue has been prepared to be used before measuring the shape of the sample. As the TM has an irregular shape, it is placed with a particular orientation reducing the shadows to a minimum. The optimum illumination direction is usually determined by a trade-off between the maximum sensitivity and minimum shadows in the reconstructed image. The size and shape of the TM are highly different from one sample to another due to breed, age and any pathology that the tissue has. Figure 4 shows the experimentally found shape of the tympanic membrane and surface profile (a), and (b) the

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shape given as a mesh data set that may be used to simulate the behavior of the tissue employing for instance finite element modeling (FEM). To measure the depth of the tympanic membrane, we consider the umbo as the reference point with respect to the tympanic annulus plane.

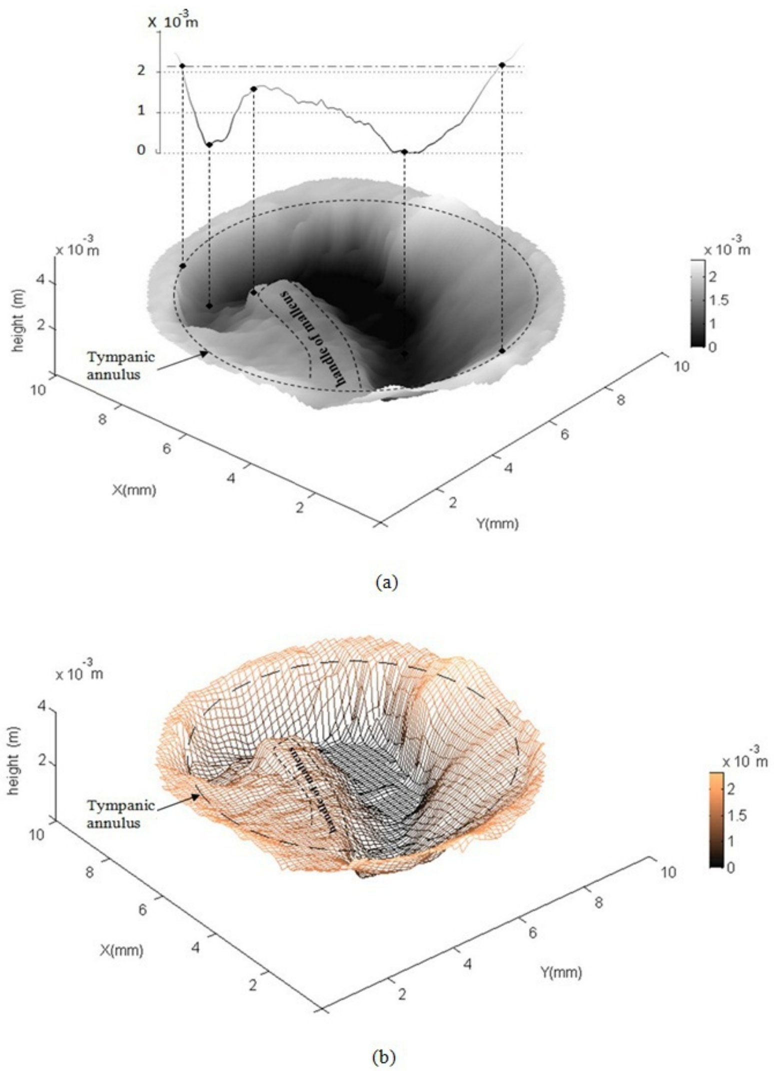

Fig. 4. Experimental results of the tympanic membrane contour. (a) 3D reconstruction and surface profile. (b) Mesh data shape.

4. Conclusions

Digital holographic interferometry combined with the two positions of object illumination method was used to measure the contour of the tympanic membrane, to the best of our knowledge reported for the first time in the literature. The shape found depicts accurately the TM surface contour, whose data can be employed to simulate its physiological behavior. DHI has shown again its usefulness to obtain contours of small and delicate biological tissues. Noninvasive rapid measurements of surface shape were performed in this work, making of this method a very useful one for medical applications.

It is a must to include the TM shape in surface deformation measurements in order to generate an accurate description of any displacement representation, being one or three dimensional. The experimental results could be used to conduct a quantitative analysis such as the measurement of displacements, obtaining in this manner more accurate values with the same setup and no additional hardware. The sensitivity of the experimental setup was fixed to an optimal angle.

Knowing the shape of the TM has an ontological significance because its topography is directly related with the ear's hearing function. The measurements made with this method could be useful to explore structural issues on surface sample thus aiding in making a better quantitative pathological diagnosis.

Measurements of biological tissues surface shape and their deformation are very important for biomechanical design, noninvasive inspection and TM its mathematical modeling.

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