3D displacement of the middle ear ossicles in the quasi-static pressure regime using new X-ray stereoscopy technique

Reference:
Salih Wasil H.M., Soons Joris, Dirckx Joris.- 3D displacement of the middle ear ossicles in the quasi-static pressure regime using new X-ray stereoscopy technique
Hearing research - ISSN 0378-5955 - (2015), p. 1-9
Full text (Publishers DOI): http://dx.doi.org/doi:10.1016/j.heares.2015.12.003

3D displacement of the middle ear ossicles in the quasi-static pressure regime using new X-ray stereoscopy technique

Wasil H.M. Salih[a,b,*], Joris A.M. Soons[b], Joris J.J. Dirckx[b]

[a] *Department of Biomedical Physics, Alneelain University, Algamhouria Avenue, Khartoum, Sudan.*
[b] *Laboratory of Biomedical Physics, University of Antwerp, Groenenborgerlaan 171, 2020 Antwerp, Belgium.*

Abstract

A novel X-ray stereoscopy technique, using greyscale information obtained from moving markers, was used to study the 3D motion in both gerbil and rabbit middle ear ossicles in the quasi-static pressure regime. The motion can be measured without visually exposing the ossicles. The ossicles showed non-linear behaviour as a function of both pressure and frequency. For instance, about 80% of the maximum umbo displacement occurs at a 1 kPa (peak-to-peak) pressure load, while a limited increase of the amplitude is noticed when the pressure goes to 2 kPa. In rabbit the ratio of stapes to umbo motion amplitude was 0.35 for a pressure of 2 kPa (peak-to-peak) at 0.5 Hz. From two stereoscopic projections of the marker paths, 3D motion of the ossicles could be calculated. This motion is demonstrated on high-resolution computer models in order to visualize ossicular chain behaviour.

*Corresponding author.
Email addresses: `wasil.hashim@uantwerpen.be` (Wasil H.M. Salih), `joris.soons@uantwerpenbe` (Joris A.M. Soons), `joris.dirckx@uantwerpen.be` (Joris J.J. Dirckx)

Keywords: Ossicular chain motion, X-ray stereoscopy, Transfer function.

1. Introduction

The middle ear (ME) is subject to different pressure fluctuations, high or low in amplitude and fast or slow in the change rate. These have been reported to happen in daily lives, and to have an influence on hearing thresholds. Therefore, several researchers have studied the deformation of the tympanic membrane (TM) and the displacement of the ME ossicles as a function of pressure using different approaches in different species. Moiré interferometry was one of the popular methods to study the TM deformation in: gerbil [6, 7, 26], cat [19], and human [5]. Moiré interferometry allows full field deformation measurements, but it takes several seconds to measure the deformed TM shape at a single pressure. Therefore, it cannot be used to measure dynamic deformation. Moreover, it requires the object to be fully visible to permit projection of a regular grid (usually a line grating) onto the object and recording of this image from a different view, which increases the dehydration of the specimen and introduces other artefacts. Another way to quantify the ossicles vibration/motion is the Mössbauer technique, which is used for ossicles motion [18], as well as for studying the vibrations of the inner ear (IE) [14, 17, 12, 25].

Apart from those two techniques, Hüttenbrink [15] used a microscope equipped with radiographic magnification to visually study the mechanical behaviour of the ossicular chain in human under static pressure. Although he could study the 3D displacements of malleus, incus, and stapes, his approach could not study the effects of the dynamic pressure changes on the ossicles, since the pressure had to be kept at the same (static) value over a long period of time. Nevertheless, Hüttenbrink presented an important detailed study that facilitated the understating of ossicular chain behaviour in the static pressure regime [15].

Recently, Gea et al. [11] studied the TM deformation in human and gerbil using the CT technique under static pressure, and thus derived the 3D displacement of the ossicles from the deformation of the TM [11]. Their method is only applicable under static pressures. Nevertheless, it requires a full scan of the ear and intensive back projection algorithms per pressure. However, in their approach, a complex image processing is required to obtain motion information.

Despite its importance to understand how the ME behaves in such pressure regimes, the 3D ME mechanics as a function of quasi-static pressure changes have not been investigated thoroughly due to practical limitations. Nevertheless, Dirckx and Decraemer [6] and Dirckx et al. [4] have studied the displacement of a gerbil umbo and a rabbit umbo and stapes respectively. In both studies, the displacement as a function of several quasi-static pressure changes were studied using high-resolution moiré interferometry for gerbils, and laser Doppler vibrometery (LDV) for rabbits. The LDV, which has become a popular technique to study the ME motion, allowed them to quantify umbo and stapes displacement along the direction of maximum displacement. But since LDV only measures displacements long one rotational axis, it cannot offer 3D displacement information, unless three laser beams from different views are used.

Vorwerk et al. [27] used a high speed camera to study the deformation of the human TM in the quasi-static pressure regime, thus the displacement of the malleus umbo could be obtained. However, like LDV the 3D motion is not achievable with this approach.

In this paper, the X-ray stereoscopy technique that we developed earlier in our papers Salih et al. [24, 23] is used to study the 3D motion information of the ossicular chain of intact rabbit and gerbil ears as a function of quasi-static pressure changes. Understanding the behaviour of the ME under such circumstances broadens our knowledge about the ME functionality, especially on how the ear deals with large quasi-static pressure loads and how the IE is protected from large pressure variations at very low frequencies [16].

Although the deformation of the TM and the displacement of the malleus ossicle have been widely studied in the static (and quasi-static) pressure regime [15, 5, 26, 6, 20, 19, 11], the transferred motion to the cochlea has been reported only in a few studies. For instance, Dirckx et al. [4] have reported the transfer function in a rabbit in the quasi-static pressure regime. However, their approach requires that the cochlea has to be opened in order to make the stapes footplate reachable for the LDV laser beam, thus the measurement is not performed in intact ears. Gan et al. [10] have reported the transfer function in human using LDV but in the acoustical domain.

When the motions of both malleus and stapes ossicles are obtained simultaneously, as is the case using the X-ray stereoscopy technique in which multiple points can be studied at the same time, the real-time transferred motion toward the cochlea can be resolved. This leads to a better understanding of the complex ME biomechanics.

2. Materials and method

2.1. Sample preparation

Adult rabbits and gerbils were used to measure the displacement of the ossicles. Gerbils were euthanised using carbon dioxide, while rabbits were sacrificed using intravenous injection of sodium pentobarbital 60 mg/kg (Dolethal, Ethical Agents Ltd). The injection was performed in the vein of the pinna after local surface anaesthesia with lidocaine spray (Xylocaine, AstraZeneca). All manipulations were conducted according to the rules set by Belgian legislation and the local ethical committee of the University of Antwerp, and were in accordance with the Guiding Principles for Research Involving Animals and Human Beings as adopted by the American Physiological Society.

Next, the temporal bone was dissected from the skull. A 3 cm long plastic tube was glued (LOCTITE® 401™) to the ear canal (EC), through which an air pressure was applied, which moves the TM and thus the ME ossicular chain. At the medial side of the bullae, a small opening was created in order to place $\approx 40\ \mu$m diameter tungsten beads, which are used as marker points. Three beads were placed on the malleus handle; one at the umbo and two further on the manubrium toward the lateral process. Two beads were placed on the stapes; one bead at each crus, cf. figure 1. The preparation was performed under a surgery microscope (Zeiss OPMI Sensera S7) to avoid any damage to the internal structures. Some beads were also placed on the promontory to assure that the specimen did not move during the measurement and the displacements of the marker points on top of the ossicles are thus exclusively due to air pressure. Moreover, a registration of the beads position before and after conducting the measurements was done to double check that the beads didn't move from their positions on the ossicles during the experiments. However, the beads adhered to the wet ossicles and promontory due to surface tension, thus no glue was needed. Please note that the bead didn't affect the dynamics of the ossicles since its weight (around 6.5 μg) is negligible compared to the weight of the ossicles, e.g. the tip of a gerbil malleus with the dimensions of: 600 μm (width) $\times$ 500 μm (height) has a weight of about 0.1 mg.

During preparation, the specimens were kept humid by working under a jet of mist of an ultrasonic humidifier (Bonaire BU-1300) directed via a plastic tube onto the specimen. After preparation, the specimens were wrapped in a wet piece of paper towel and placed in a container made of thin acrylic tubing, to avoid dehydration of the tissue structures, cf. figure 2.

The measurement protocol with moistening that has been followed allowed to do the measurements in fresh samples in order to reduce the post-mortem artifacts, since the measurements were finalized in 1.5 hours after sacrificing the gerbils and within 3 hours for rabbit. The latter took longer as the measurements had to be done in a CT facility 60 km away from the laboratory, where the specimens were prepared. Moreover, some pressure cycles were applied before conducting the measurements as preconditioning process, which can reduce the artefacts due to viscoelastic properties of the membrane. Preconditioning is a biomechanical phenomenon, in which that tissue behaviour changes due to repetitive loading-unloading experiments [9]. Funk et al. [8], have studied the differences between preconditioning and unpreconditioning processes for ankle ligaments. They concluded that pre-

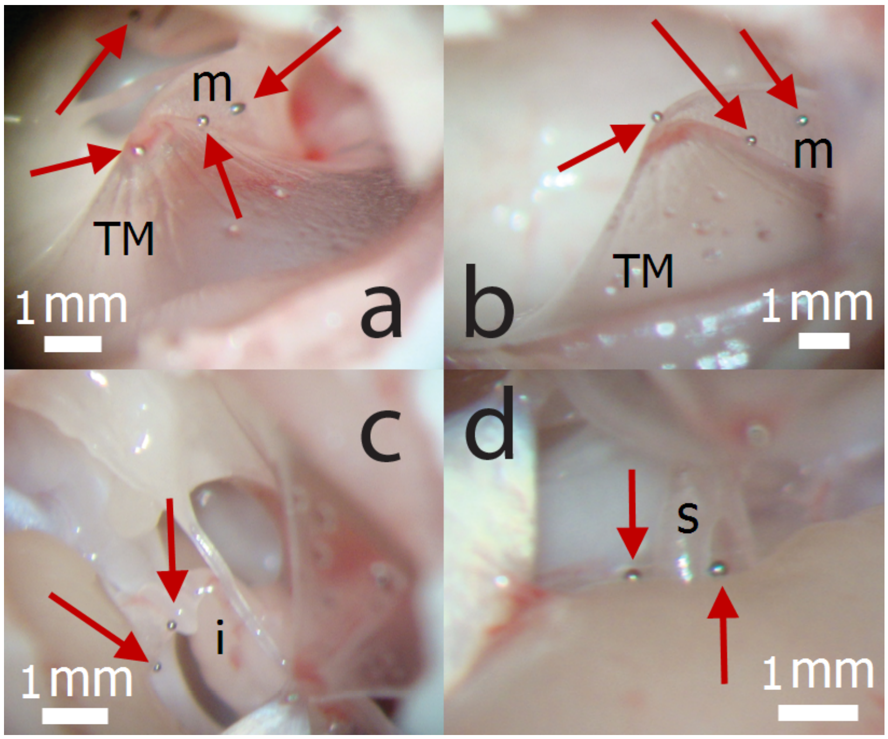


Figure 1: Snapshots of beads on top of rabbit ossicles taken under a surgical microscope: (a) arrows indicate three beads on a rabbit malleus and one (top left) on the incus, (b) zoomed in for the beads on the malleus, (c) one bead on the incus and one on the stapes crura and (d) one bead on the stapes crura and other one on the promontory, which has been used to assure that the specimen did not move during the measurement. TM: tympanic membrane, m: manubrium, i: incus and s: stapes.

conditioning affected the short-time behaviour of ligament relaxation, but not the long-time behaviour [8]. In hearing research, several studies have demonstrated this phenomenon, such as Gaihede [9], Aernouts and Dirckx [2]. Gaihede [9] has studied the tympanometric preconditioning. He reported that the preconditioning process leads to more stable results of TM response to pressure loads. Therefore, we decided to precondition our specimens before start conducting the measurements.

2.2. Pressure generation

A custom-built pressure generator was used to apply a uniform dynamic air pressure to the EC. The pressure setup consisted of an electromagnetic actuator (Frederiksen 2185.00) that was attached to an adaptable gas volume in connection with a tube. When the actuator moves, the pressure changes, since the amount of gas remains constant. With a pressure sensor (Druck PDCR 10/L) coupled to the tube, the exact pressure values were obtained using a custom-built feedback system [1].

A function generator (Tektronix TDS 210), attached to the feedback system, was used to generate the frequency of the desired pressures within the range of ± 2 kPa at frequencies varying from 0.1 to 100 Hz. In this paper, pressures of 1 and 2 kPa (peak-to-peak amplitude) at frequencies of 0.5, 1, 5, 10, 20, 30, 40 and 50 Hz were used.

2.3. Measurement of the motion

As presented in our papers [24, 23], a method has been developed to study the 3D motion of the internal structures of an object. It makes use of

X-ray stereoscopy in order to obtain the 3D motion information within non-transparent objects, such as the ME, from greyscale variations. In short, the method works as follows: Using the X-ray point source of a CT machine, two images are recorded from two different directions (by rotating the object over 90^o between imaging), while the internal structures are moving. The 3D coordinates of marker points in the world coordinates system can be obtained from the coordinates of the marker points in the camera coordinates system in the pair of images. When the 3D coordinates of the two outer points of the displacement are calculated, the amplitude of motion for a periodically moving object can be measured.

Since the ossicles moved during the X-ray imaging due to the applied air pressure, the integrated recorded intensity along the path of motion of the marker points, which have been placed on top of the ossicles, showed different values due to the motion speed. When a marker remains longer in a given position, more X-rays will be absorbed and vice versa., cf. figure 3. Therefore, the greyscale of the X-ray shadow image can be used to reconstruct the time information by integration and thus the 3D motion information can be obtained.

The measurements were performed as follows: The gerbil specimens were placed in the specimen holders of a μCT (skyscan 1072) with a spot size of 8 μm, while the rabbit specimens were imaged using a custom built state-of-the-art μCT (UGCT -Ghent University), which can achieve feature recognition of 2 μm, as specified by the X-ray tube manufacturer [21]. The latter was used since rabbit bone is more dense, thus more X-ray energy is needed to distinguish between the tungsten beads and the bone.

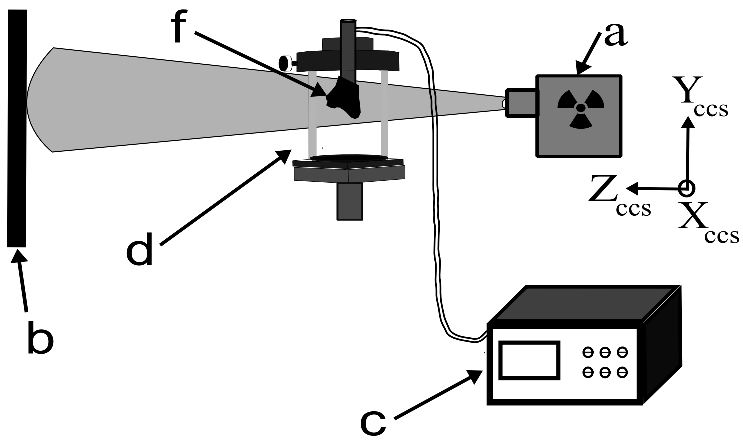


Figure 2: A schematic drawing of the X-ray stereoscopy setup to measure a dynamic displacement in gerbil and rabbit ears: (a) X-ray point source, (b) detector, (c) pressure generator, (d) specimen holder that rotates over angle β and (e) specimen.

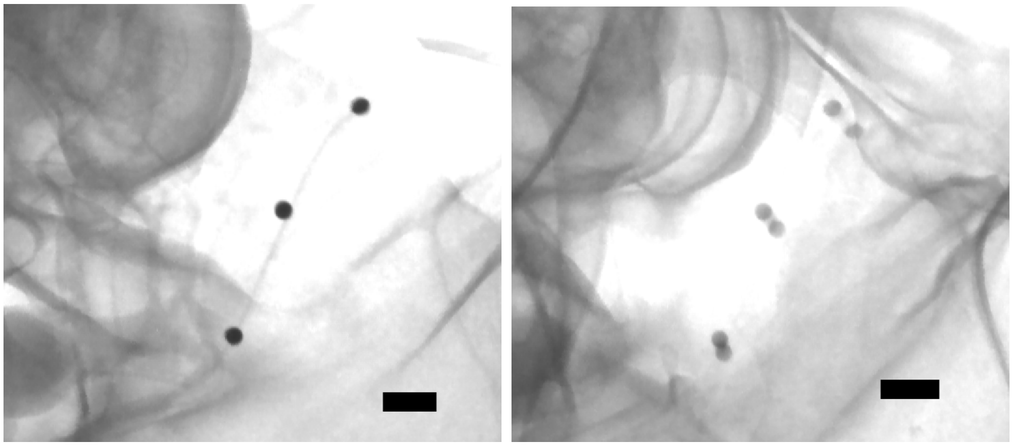
Next, a pair of images with 90^o separation angle was recorded with exposure time of 10 s during excitations with 1 and 2 kPa (peak-to-peak) at 0.5, 1, 5, 10, 20, 30, 40, and 50 Hz. This exposure time has been chosen to be an integer number of periods of the movement. After the measurement session, a full CT scan was performed in order to generate a computer model of the ossicles with beads, which will be used later to demonstrate the motion. The displacement of the marker points is registered to the ossicles computer model using the aligning function in *Amira*® 5.3.3 (Visage Imaging).

3. Results

3.1. Gerbil ear

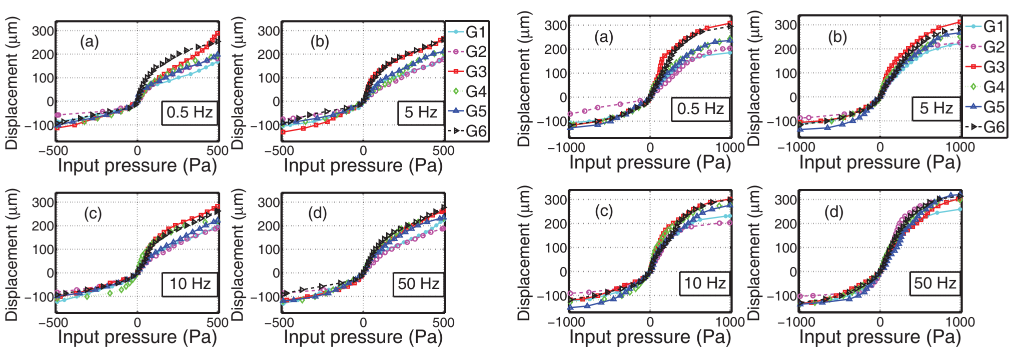
An example of the tungsten beads movement during sinusoidal stimulation is presented in figure 3 in order to show how the greyscale varies between rest (figure 3(a)) and excited state (figure 3(b)). The figure shows that at the two outer points, more X-rays were absorbed as the ossicles motion becomes slower (the bead spends more time here) than in the middle of the motion event, where the bead moves faster and thus absorbs less X-rays.

The displacement amplitudes of 6 gerbil umbos as a function of 1 and 2 kPa (peak-to-peak) at frequencies of 0.5, 5, 10 and 50 are presented in figure 4. For a pressure of 1 kPa (peak-to-peak), the gerbil umbos show an average amplitude of (307 ± 40) μm at 0.5 Hz, which increases to (348 ± 28) μm at 50 Hz. The amplitudes of other frequencies lie in between. When a bigger pressure is applied to the gerbil ears (2 kPa), the amplitude increase to values between (354 ± 42) μm at 0.5 Hz and (428 ± 26) μm at 50 Hz, cf. figure 5.



(a) (b)

Figure 3: Snapshots of X-ray shadow images show three tungsten beads on top of the malleus ossicle of a gerbil at: a) rest state before applying a pressure and b) during linear loading of the tympanic membrane with a pressure of 2 kPa (peak-to-peak) at a frequency of 50 Hz. The scale bar is 500 μm.



(a) (b)

Figure 4: Displacement of gerbil umbos as a function of: (a) 1 kPa and (b) 2 kPa, at frequencies of: 0.5 Hz, 5 Hz, 10 Hz and 50 Hz.

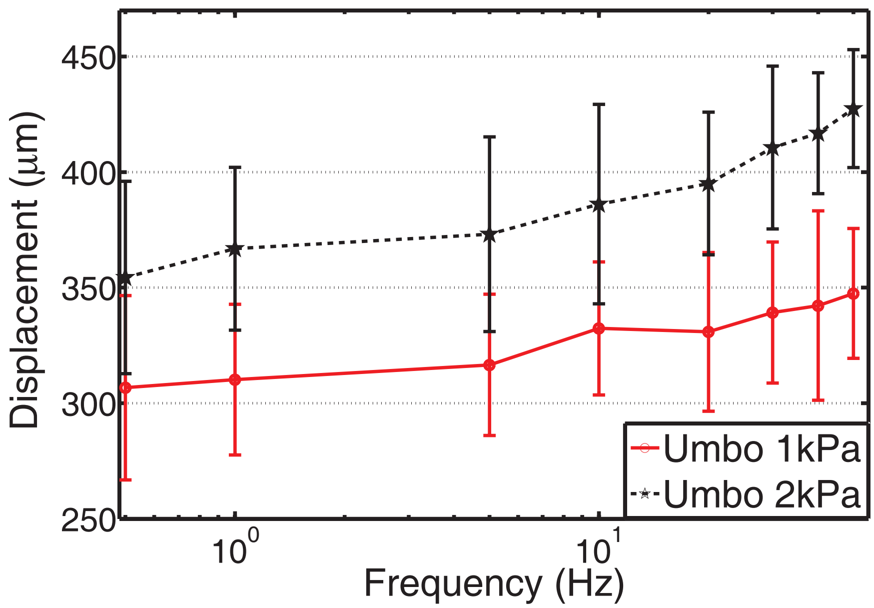
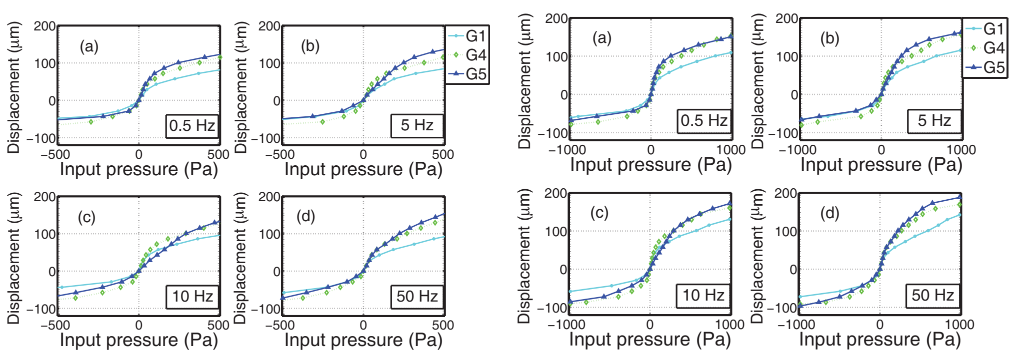


Figure 5: Average displacement amplitude of gerbil umbos as a function of 1 kPa (red solid line) and 2 kPa (dashed black line).

Due to practical limitations, it was not possible to reconstruct the path of motion for all of the beads that were placed toward the lateral process of the malleus manubria. Nevertheless, the amplitudes of motion for all mid manubrial beads have been obtained from stereoscopy. The manubria displacements as a function of 1 kPa (peak-to-peak) show values between (162±17) μm at 0.5 Hz and (205±16) μm at 50 Hz. When 2 kPa (peak-to-peak) is applied, the displacement at 0.5 Hz shows a value of (213±26) μm, while this value increases to be (254±24) μm at 50 Hz.

3.2. Rabbit ear

Since the dimensions of the rabbit ossicles are bigger than in gerbils [22], the positioning of beads becomes easier, which facilitates the accurate measurement of the umbos, manubria (2 places) and stapes displacements. Figure 6 shows the displacements of 6 rabbit umbos as a function of peak-to-peak pressures values of 1 kPa (figure 6(a)) and 2 kPa (figure 6(b)).



(a) (b)

Figure 6: Displacement of rabbit umbos as a function of peak-to-peak pressures values of: (a) 1 kPa and (b) 2 kPa, at frequencies of: 0.5 Hz, 5 Hz, 10 Hz and 50 Hz.

The average displacement amplitudes of the umbos, manubria and stapes with their standard deviations are presented in figure 7. This figure shows clearly that the maximum displacement is performed by the umbo while the stapes movement is the smallest.

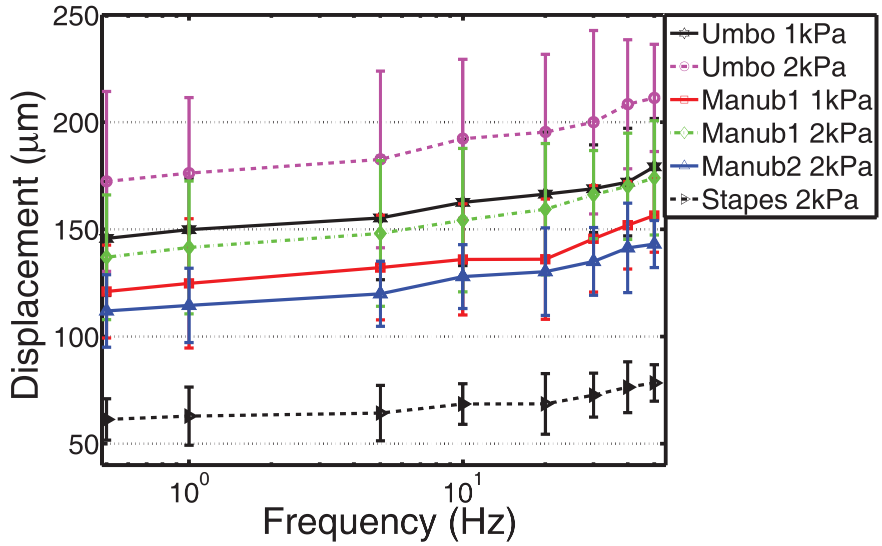
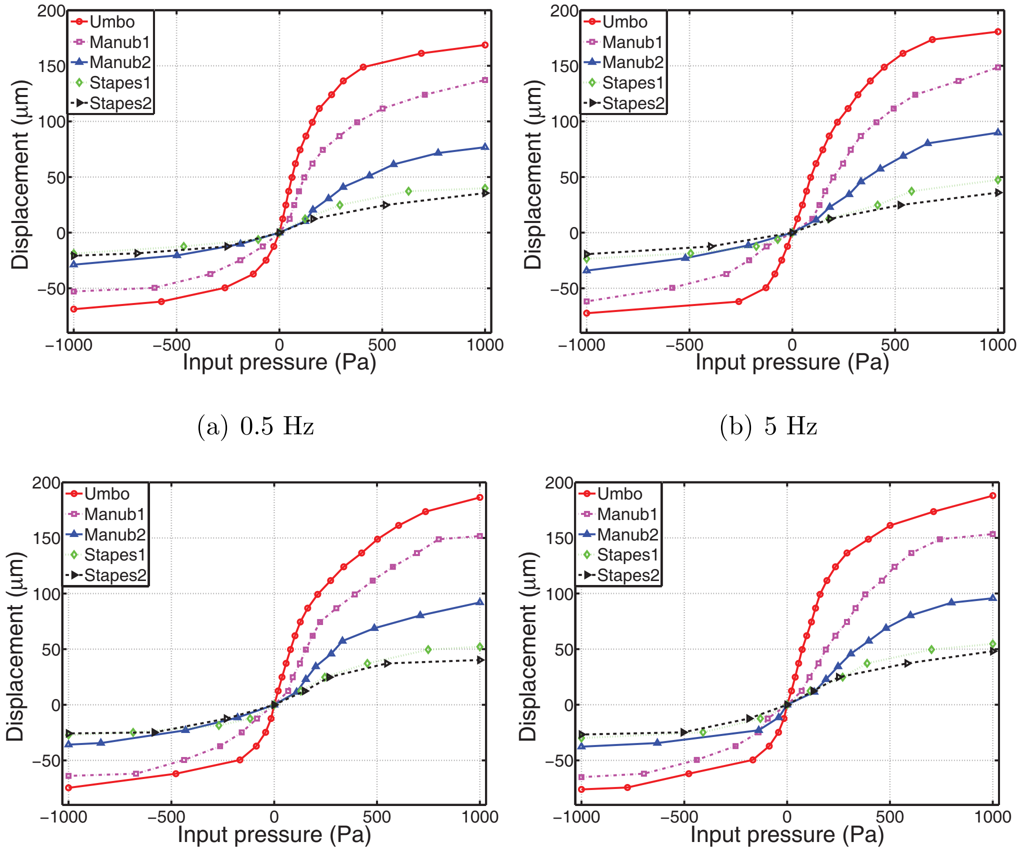


Figure 7: Average amplitude of rabbits' umbos, manubria and stapes s as a function of 1 kPa and 2 kPa (peak-to-peak).

To facilitate the comparison between the ossicles displacements, figure 8 presents displacements of the umbo, manubrium (at two positions) and stapes (one at each crus) of specimen #R4 as a function of 2 kPa (peak-to-peak) at different frequencies.

3.3. 3D displacement

As mentioned before, full scans of the specimens have been made in order to generate computer models of the gerbil and rabbit ears, including the



(a) 0.5 Hz

(b) 5 Hz

(c) 10 Hz

(d) 50 Hz

Figure 8: Displacement of umbo, manubrium (at two positions) and stapes (at the two crus) of a rabbit (specimen R4) as a function of 2 kPa (peak-to-peak) at frequencies: (a) 0.5 Hz, (b) 5 Hz, (c) 10 Hz and (d) 50 Hz.

beads. These models are required to represent the 3D displacement of the ossicles, since the coordinates of the beads - relative to the ossicle - are needed to show the motion. From 180 projections (one per 1^o), a back projection is calculated, and a set of virtual slice images of the object is obtained. These slices are then segmented and the 3D shape of the object is reconstructed. If the internal structure has the full 6 degrees of freedom, then 3 markers are needed to determine its full three-dimensional motion.

The malleus mainly moves along one direction and at low frequencies it rotates along a certain axis [3]. Therefore, two beads suffice to measure the rotational movement. It has been noticed that the beads strongly affect the reconstructed images since the reconstruction algorithm is based on Lambert-Beer law, which doesn't work properly for a very dense materials as the case with the tungsten. These low-resolution reconstructed images thus affect the segmented/created computer models, which make them less useful to demonstrate the motion event. To tackle this problem, high-resolution morphological computer models of the MEs of gerbils and rabbits that have been developed earlier [22], were used for the 3D representation. The models including the beads were registered with the high-resolution models in order to demonstrate the motion on this better quality representation.

The 3D displacements of the marker points, which were placed on the gerbil malleus and rabbit malleus and stapes, were applied to the models in order to represent the ossicles displacements. In a gerbil, just the motion of malleus ossicle can be shown, while in a rabbit displacements of both malleus and stapes ossicles can be shown. The motion of malleus and incus is mainly a rotation, and therefore linear motions can be measured best at points far from the rotation axis. We also need to be able to position the beads, which proved to be not possible on some parts of the incus as they are hidden under the bone. Therefore motions were measured using beads on the manubrium at positions some distance away from the axis of rotation and which could be reached to position the markers. We did not manage to put a marker perfectly at the tip of the incus, but we could place it on the stapes crura. Within the measuring resolution, incus and stapes will move together.

Figure 9 shows superimposed snapshots of the 3D reconstruction of the malleus ossicle of a gerbil in its two extreme positions, when pressure between -1 kPa and +1 kPa is applied at a frequency of 50 Hz. The shape of the (considered) rigid structure is obtained from the tomography. The position of the ossicle was determined from the stereo projections of the marker points (the two spheres, one at the umbo and the other at the manubrium). Using the new greyscale analysis technique, time information of the motion as a function of pressure is obtained. This can, of course, not be shown in a single image.

The same for rabbits is presented in figure 10. The figure shows superimposed snapshots of the reconstructed 3D motion of the ossicular chain when 2 kPa (peak-to-peak) is applied to the TM at 50 Hz. From the figure, one sees that when the umbo moves in the lateral side (at + pressure), the stapes goes in the medial side toward the cochlea, and vice versa.

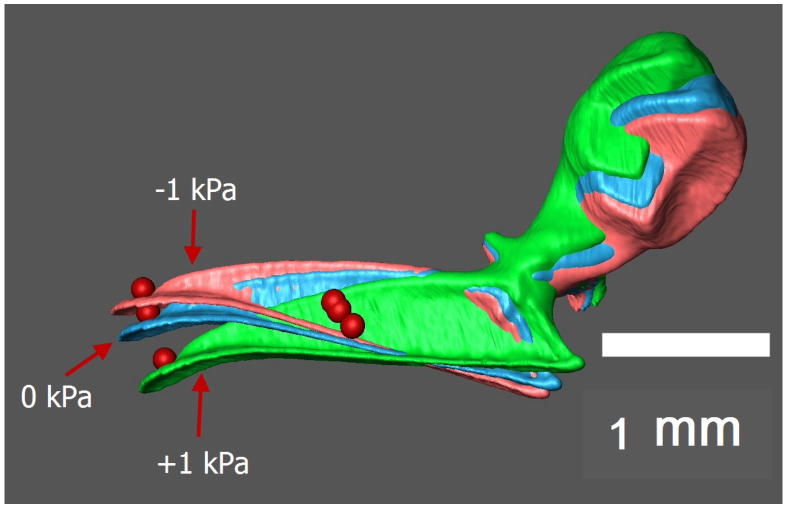


Figure 9: Superimposed snapshots of the 3D motion of the malleus of a gerbil ME during pressurizing the EC with 2 kPa (peak-to-peak) at a frequency of 50 Hz. The beads are represented by spheres that are out of the scale.

4. Discussion

4.1. Measurement setup

Unlike the moiré interferometry and LDV techniques, X-ray stereoscopy does not require the internal structures/features under study to be visually exposed. Moreover, the 3D displacements for the ossicles are obtained from just one pair of images recorded in few seconds. This approach uses one single X-ray point source with the object rotated in between the recording of a pair of images, so no need for two X-ray sources as in classical X-ray stereoscopy. By setting the exposure time to an integer number of periods of the movements, the displacements of the ME ossicle as a function of dynamic sinusoidal pressure changes of frequencies between 0.5 and 50 Hz and peak-to-peak amplitudes of 2 kPa can be studied in less than 30 s, which is not

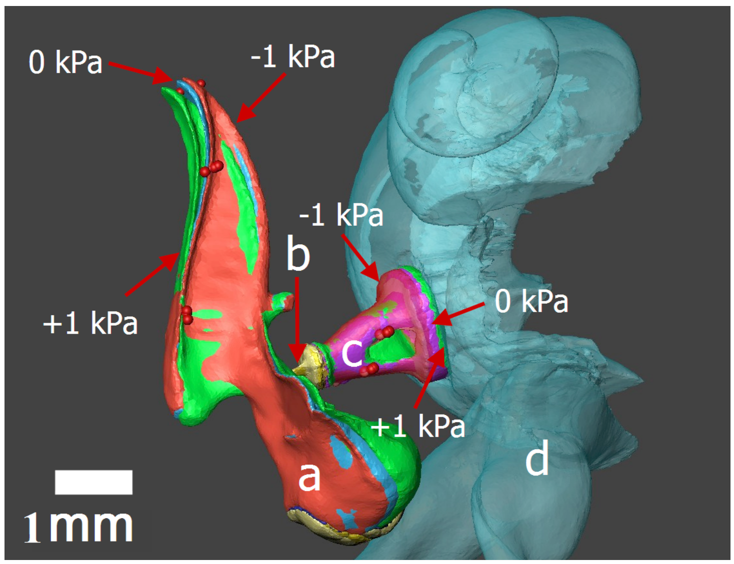


Figure 10: Superimposed snapshots of the 3D motion of a rabbit ossicular chain during pressurizing the EC with 2 kPa (peak-to-peak) at a frequency of 50 Hz. Arrows indicate the displacement as a function of pressure. (a) is the malleus, which contains 3 beads, (b) is the incus, it is displaced as a part of the malleus, (c) is the stapes, which has two beads one at each crura. The stapes moves inward (at + pressure) and outward (at - pressure) the cochlea (d), which does not move as a function of pressure. The beads are represented by spheres that are out of the scale.

achievable with moiré interferometry, or with a full CT scan.

The greyscale variation of the X-ray shadow images in 2D marked the motion of the bead along the 3D (approximately linear) path of the ossicles motion. Combined with the 3D coordinates, calculated from stereoscopy, the 3D displacement can be demonstrated on computer models, which helps to identify the behaviour of the ossicular chain in such pressure regime. Another advantage of the current method is that the displacement at several points on the ossicles can be achieved simultaneously; unlike LDV where one point per laser beam can be studied.

Figure 3 shows an example of X-ray shadow images. At rest state, tungsten beads have approximately the same greyscale value. From these, the size of the beads is determined, which is used to set the size of the Gaussian filter that is used to obtain the greyscale values [23]. Using smaller beads will improve the measurement accuracy, but there are practical issues that determine the appropriate bead size. On the one hand they need to be manipulated on the ossicles, on the other hand they need to absorb enough X-rays.

Figure 3(b) shows how the displacement of the bead at the umbo is larger than that of the bead close the lateral process of the malleus. Moreover, it shows that they move in relative different directions. In addition, one sees that there is a variation in the background intensity around the beads; the bead in the middle has a brighter background than the other two. The background of the bead at the umbo, the upper one, is not homogeneous. Therefore, first order correction for changes in the background intensities was needed. Consequently, the greyscale of each bead is calculated individually using the actual background intensity in order to accurately reconstruct the path of motion.

When a faster motion event (high frequency) is studied, more accurate results are expected than in a slower motion (low frequency), if the exposure time remains the same in the two cases. This is because a marker moving at higher frequency crosses the same points more times than at lower frequency, thus the recorded intensity is averaged over more periods which leads to a more continuous variation in the greyscale. Nevertheless, even in a very low frequency, exposure time can be set to ensure tens of periods depending on the ability of the X-ray machine.

As discussed before, one can determine the 3D position of the markers in their most outward positions, and determine the amplitude of displacement. In principle, this information does not suffice to quantify any general motion, as the exact position of the marker at each time point is unknown. It is, for instance, not possible to determine the phase of the motion; one can only determine the magnitude of the displacement along a linear path of motion. In most applications, however, the high-resolution method will be mainly intended to measure small movements, which are to a good approximation along a linear path, making fully generalized motion measurement less of an issue. However, the method is not limited to be used just in ME research, it can find its way in other biomechanics applications since it offers fast 3D motion measurements within opaque objects.

4.2. Ossicles displacement

As shown in the results, all specimens exhibited similar behaviour for the ossicles displacements as a function of pressure. However, one can see some variations in the displacement amplitude. This can be attributed to several reasons such as interspecies variation or the positioning of the beads on top of the ossicles (it is practically impossible to place the beads at the same position in different specimens). The pixel size cannot be set to the same value for all specimens due to a practical reason: the orientation of the specimen is varied between recordings to ensure the best view. Nevertheless, the average displacement amplitude of the ossicles in gerbils and rabbits, showed a standard deviation of less than 45 μm, which is equivalent to about 6 pixels.

The displacements of the ossicles in both gerbil and rabbit showed a sigmoid or S-like shape, meaning that the displacement as a function of pressure increases fast at the beginning, while it increases much slower when higher pressure is applied. Consequently, most of the displacement happened while pressure varied between -500 Pa and +500 Pa. Pressures of 1000 Pa (peak-to-peak) produced displacements which have a magnitude of 80% of the ossicles displacements. For instance, rabbit umbos show an average displacement amplitude of (179$\pm$23) μm for a pressure of 1 kPa (peak-to-peak) at 50 Hz. This value increases by about 40 μm to (211$\pm$25) μm when the pressure increases to 2 kPa (peak-to-peak), which means that approximately 80% of the displacement occurs below/at 1 kPa. This shows clearly that the ossicles have a strong nonlinear behaviour as a function of pressure, which has been reported earlier by Dirckx et al. [4].

From the results section, one sees that the displacements of the ossicles show bigger values when pressure cycles of higher frequency were applied. The displacement in both gerbil and rabbit umbos increase by about 73 μm and 38 μm, respectively, when the frequency increases from 0.5 Hz to 50 Hz for the same pressure amplitude. This shows that the ossicles move less when a static and quasi-static pressure changes were applied than when frequencies approach the acoustic domain. Nonetheless, the ME is known to act as a high pass filter protecting the IE from excessive pressure changes. This protective function has been observed in human [15]. It was mainly attributed to the sliding motion between the malleus and incus. In rabbit and gerbil, the incus and malleus are fused as one ossicle, but we also see that ultra-low frequencies cause less stapes motion than acoustical frequencies. Additionally, when a positive pressure is applied to the EC (negative ME pressure), smaller displacements for the ossicles are observed than for a negative EC pressure (positive ME pressure) [4, 6]. This effect can, at least, partly be attributed to the conical shape of the TM, making movements towards the ME (medially) more difficult than inflation movements towards the EC (laterally).

In gerbils, the umbos show an average amplitude of (428$\pm$26) μm at 2 kPa, 50 Hz, while the beads at the manubrium toward the lateral process show an average amplitude (254$\pm$24) μm. This shows that the displacement drops by more than 40% between the two measurement points. On average, the beads at the umbo measure about 200 μm to 300 μm from the tip of the manubrium, while the beads further toward the lateral process have been placed from 1050 μm to 1200 μm, relatively to the beads at the umbo.

The current gerbil umbos show maximum amplitude at a pressure of 2 kPa of (428$\pm$26) μm while Dirckx and Decraemer have reported a value of about 410 μm for the same pressure [6]. In their paper, they measured the displacement as at pressures up to 4 kPa, and they found a value of 460

μm, which confirms that limited displacement can occur with increasing the pressure to the more than 1 kPa. Gea et al. [11] have reported a limit value of 294 μm. This shows a difference of more than 100 μm between the current results and the one obtained by Gea et al. [11]. However, the latter is done in static pressure load, which may decrease the amplitude of motion, as it has been reported now that the displacement increases as a function of frequency.

In rabbits, the results show average umbo displacements of (211$\pm$25) μm while Dirckx et al. [4] have reported (165$\pm$19) μm. Stapes displacements were found to be (78$\pm$9) μm with the X-ray stereoscopy approach. Moreover, Dirckx et al. [4] have reported a limited value of (34$\pm$5) μm. One can notice that the current results show a relatively bigger displacement for the ossicles. This can be attributed to the requirements that LDV should exactly measure along the direction in which the object under study has its maximum displacement. Due to practical issues, this is always a challenge because of the anatomical structure of the ME. Therefore, the displacement might be smaller. Whilst in X-ray stereoscopy, one only needs to place the specimen in the path of an X-ray bundle and rotate the object to have the best view. Moreover, the displacement is measured from the 3D coordinates of the marker points; therefore, the motion in all directions is taken into account. However, interspecies variations as well as the position of the marker point, relative to the ossicles, are also sources of variability.

4.3. 3D motion

As the stereoscopy technique offers the 3D coordinates of the two outer points of a motion event, and as the greyscale variety of X-ray shadow images can be used to obtain time information, the 3D motion of the beads and thus the ossicles are obtained on a linear path. Moreover, they can be shown in a 3D computer model. In gerbils, it was only possible to present the motion for the malleus ossicle, whilst in rabbits the motion of stapes ossicle could be presented as well. The displacement of the incudo-mallear complex was treated as one rigid body using three beads, one at the umbo and two further at the manubrium toward the lateral process. It is not possible to present the 3D displacement in a single image, so, snapshots of these motions are presented in figure 9 and figure 10 in order to give a reader an idea about how the ossicles move.

4.4. Transfer function

The contribution of the ME ossicles to transfer the motion from the TM toward the cochlea is well-known as one of the ME functions. However, few efforts have been made to identify this action in the quasi-static pressure regime. In the current study, the displacements of the malleus and the stapes ossicles in rabbits have been measured simultaneously. In this way, the real time transfer function is achieved, which leads to a better understanding of the behaviour of the ME in such circumstances.

As seen in the results section, the motion transfer from the malleus to the incus shows non-linearity as a function of both pressures and frequencies. When 2 kPa (peak-to-peak) with a frequency of 0.5 Hz is applied to rabbit ears, the ratio of stapes, (61$\pm$10) μm, versus malleus, (172$\pm$42) μm, displacements show a value of 0.35. The ratio is increased a bit to 0.36 at 50 Hz ((78$\pm$9) μm for the stapes and (211$\pm$25) μm for the malleus). This shows that it is the ratio that remains practically unchanged, even under high pressure, while the displacement increases with frequency.

This ratio is very close to the lever arm value that is reported in rabbit (0.4) [13]. However, it is bigger than the ratio (0.2 to 0.3) that has been reported by Dirckx et al. [4]. The latter can be attributed to the fact that Dirckx et al. [4] measured the footplate motion without the fluid load of the cochlea, while the current measurements have been done in intact ears [4].

4.5. Hysteresis

In previous work, using laser vibrometry, it has been shown that both umbo motion and stapes motion shows hysteresis at very low motion frequencies [4]. Although it was shown that hysteresis is mainly present for pressure change rates lower than 1 kPa/s, some hysteresis may be present in the movements we measured in this paper. In the current technique we measure the average displacement path over time, so it is not possible to discriminate between the pressure increasing phase and the pressure decreasing phase. Hence, the technique does not yet allow to study hysteresis, which is a limitation of the method at this point. A possible solution could be to expose the camera only during one phase of the pressure cycle, but in order to do this the output of the X-ray source needs to be switched on and off synchronized with the pressure phase.

5. Conclusion

A setup has been developed that makes it possible to measure the 3D motion of the ME ossicles in a closed ME, without the need of visually exposing the ossicles. Pressures in the range of 0 to 2 kPa (peak-to-peak) at the frequencies of 0.5 to 50 Hz were used. The approach makes use of the combination of X-ray stereoscopy and greyscale (thus time) information, obtained from the X-ray shadow images, in order to reconstruct the 3D linear path of motion such as the motion of the ossicles motion as a function of quasi-static pressure changes.

The ossicles motion is found to show a nonlinear response to the applied pressure. The major part of the displacement amplitude occurred during pressurizing the EC with peak-to-peak pressure amplitudes of 0 and 1 kPa, and only a small increase of the amplitude is noticed between 1 and 2 kPa (peak-to-peak). Moreover, the ossicles displacements increase with frequency, which means that ME is adapted to avoid big deformation in response to the quasi-static high-pressure changes.

The ratio of stapes to umbo motion amplitude is 0.35 for a pressure of 2 kPa (peak-to-peak) at a frequency of 0.5 Hz. The ratio increases a bit to 0.36 as a function of a faster pressure change (at 50 Hz). The value agrees with the lever arm ratio that is reported in rabbits. High-resolution computer models facilitate our understanding of ME behaviour in this pressure regime.

The results show that the new method of X-ray stereoscopy, combined with greyscale analysis along the path of moving markers, opens up new possibilities to measure the ME ossicles motion in 3D. Gerbil morphology is on the edge of the resolution of the current approach, for rabbit better results can be obtained. For human temporal bones, dimensions and motions will still be larger, so relative measurement resolution will be better. Nevertheless, it will be a challenge to detect the marker beads within the strongly absorbing dense temporal bone. With more powerful X-ray point sources becoming available, the method discussed in the present work will allow new possibilities to study ossicles motion in the transition range between quasi-static and acoustic pressures.

Acknowledgements

This work was partially supported by the Research Foundation - Flanders (FWO-Vlaanderen). The authors would like to thank F Wiese and W Deblauwe for their assistance in specimen preparation, and Professor Dr N De Clerk and Ir F Lakiere for their help and putting the micro-X-ray scanning equipment at our disposal. We appreciate the use of and collaboration with the scanning facility of UGCT, Gent, Belgium.

references

[1] Aernouts, J., Dirckx, J., 2011. Elastic characterization of the gerbil pars flaccida from in situ inflation experiments. Biomech Model Mechanobiol 10 (5), 727–741.

[2] Aernouts, J., Dirckx, J., Feb. 2012. Viscoelastic properties of gerbil tympanic membrane at very low frequencies. J Biomechanics 45 (6), 919–924.

[3] Decraemer, W., Khanna, S., 1994. Modelling the malleus vibration as a rigid body motion with one rotational and one translational degree of freedom. Hear Res 72 (1-2), 1–18.

[4] Dirckx, J., Buytaert, J., Decraemer, W., 2006. Quasi-static transfer function of the rabbit middle ear, measured with a heterodyne interferometer with high-resolution position decoder. J Assoc Res Otolaryngol 7 (4), 339–351.

[5] Dirckx, J., Decraemer, W., 1991. Human tympanic membrane deformation under static pressure. Hear Res 51 (1), 93–105.

[6] Dirckx, J., Decraemer, W., 2001. Effect of middle ear components on eardrum quasi-static deformation. Hear Res 157 (1-2), 124–137.

[7] Dirckx, J., Decraemer, W., von Unge, M., Larsson, C., 1997. Measurement and modeling of boundary shape and surface deformation of the mongolian gerbil pars flaccida. Hear Res 111 (1-2), 153–164.

[8] Funk, J. R., Hall, G. W., Crandall, J. R., Pilkey, W. D., 2000. Linear and quasi-linear viscoelastic characterization of ankle ligaments. Journal of biomechanical engineering 122 (1), 15–22.

[9] Gaihede, M., 1996. Tympanometric preconditioning of the tympanic membrane. Hear Res 102 (1-2), 28–34.

[10] Gan, R., Wood, M., Dormer, K., 2004. Human middle ear transfer function measured by double laser interferometry system. Otol Neurotol 25 (4), 423–435.

[11] Gea, S., Decraemer, W., Funnell, W., Dirckx, J., Maier, H., 2010. Tympanic membrane boundary deformations derived from static displacements observed with computerized tomography in human and gerbil. J Assoc Res Otolaryngol 11 (1), 1–17.

[12] Guinan, J., Cooper, N., 1974. Medial olivocochlear efferent inhibition of basilar-membrane responses to clicks: Evidence for two modes of cochlear mechanical excitation. J Acoust Soc Am 55, 588–596.

[13] Hemilä, S., Nummela, S., Reuter, T., 1995. What middle ear parameters tell about impedance matching and high frequency hearing. Hear Res 85 (1-2), 31–44.

[14] Hillman, P., Schechter, H., Rubinstein, M., 1964. Application of mossbauer technique to measurement of small vibrations in ear. Rev Mod Phys 361.

[15] Hüttenbrink, K., 1988. The mechanics of the middle-ear at static air pressures: The role of the ossicular joints, the function of the middle-ear muscles and the behaviour of stapedial prostheses. Acta Otolaryngol Suppl 451, 1–35.

[16] Hüttenbrink, K., 2001. Middle ear mechanics and their interface with respect to implantable electronic otologic devices. Otolaryngol Clin North Am 34 (2), 315–335.

[17] Johnstone, B., Boyle, A., 1967. Basilar membrane vibration examined with the mössbauer technique. Science 158 (3799), 389–390.

[18] Kliauga, P., Khanna, S. M., 1983. Dose rate to the inner ear during mossbauer experiments. Phys Med Biol 28 (4), 359–366.

[19] Ladak, H., Decraemer, W., Dirckx, J., Funnell, W., 2004. Response of the cat eardrum to static pressures: Mobile versus immobile malleus. J Acoust Soc Am 116 (5), 3008–21.

[20] Lee, C., Rosowski, J., 2001. Effects of middle-ear static pressure on pars tensa and pars flaccida of gerbil ears. Hear Res 153 (1-2), 146–163.

[21] Masschaele, B., Cnudde, V., Dierick, M., Jacobs, P., Hoorebeke, L. V., Vlassenbroeck, J., 2007. UGCT: New X-ray radiography and tomography facility. Nucl Instrum Methods Phys Res A 580 (1), 266–269.

[22] Salih, W., Buytaert, J., Aerts, J., Vanderniepen, P., Dierick, M., Dirckx, J., 2012. Open access high-resolution 3D morphology models of cat, gerbil, rabbit, rat and human ossicular chains. Hear Res 284 (1-2), 1–5.

[23] Salih, W., Buytaert, J., Dirckx, J., 2011. Measurement of micro-motions within non-transparent objects using gray scale information in x-ray stereo projection imaging. Meas Sci Technol 22 (3), 035801, 1–11.

[24] Salih, W., Soons, J., Dirckx, J., 2011. High-resolution 3D translation measurements using point source x-ray stereoscopy. Meas Sci Technol 22, 025502, 1–7.

[25] Schairer, K., Fitzpatrick, D., Keefe, D., 2003. Input-output functions for stimulus-frequency otoacoustic emissions in normal-hearing adult ears. J Acoust Soc Am 114, 944–966.

[26] von Unge, M., Decraemer, W., Bagger-Sjöbäck, D., Dirckx, J., 1993. Displacement of the gerbil tympanic membrane under static pressure variations measured with a real-time differential moiré interferometer. Hear Res 70 (2), 229–242.

[27] Vorwerk, U., Steinicke, G., Begall, K., 1999. Observation of eardrum movements during quasi-static pressure changes by high-speed digital imaging. Audiol Neurootol 4 (3-4), 150–155.