Studying the functional morphology of the middle ear by visualizing bone and soft tissue structures with microCT

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Aims
The middle ear is a complex biomechanical system positioned between the outer and inner ear, and transfers sound energy from air to fluid by impedance matching. It forms an essential part of the ear and consists of three small bones and the eardrum. Understanding its working principles has been a quest for many researchers, and its exact functioning is still not fully understood. High realism finite-element modeling is becoming a widespread used technique in middle ear research, but highly accurate morphological input data are needed. With this work, we first of all aim to contribute to the field of middle ear mechanics, by providing the necessary input for finite element modeling. Exact morphological data is needed in 3D on the shape, location and dimensions of all middle ear structures, including ligaments and the two muscles present in the middle ear.

- Where do the suspensory ligaments attach to the hearing bones (ossicles)?
- What is the volume, shape and orientation of the muscles, ligaments, ossicles?
- Do the ossicles possess hollow internal structures and/or blood vessels?
- How are the joints shaped and held together?

These questions on 3D morphology can be answered by combining state-of-the-art X-ray micro-computer tomography with a staining procedure to make soft tissue visible in back-projected cross sections. These data required specialist manual segmentation by researchers with expert notion of human middle ear morphology.

Once we have a finite element model and simulations that are true to nature, fundamental questions about the tympanic membrane (TM) as well as clinically relevant questions can be addressed, such as:

- What is the role of the non-isotropic nature or the inhomogeneous thickness distribution of the eardrum?
- How would the vibrational pattern on the eardrum be affected if the manubrial fold (a thin soft tissue membrane between the malleus ossicle (hammer hearing bone) and the tympanic membrane (eardrum)) was absent or when the eardrum was rigidly connected to the manubrium?
- How does the vibrational pattern change when the eardrum is under a static pressure load?
- What happens in the clinically interesting case of eardrum perforation or a pressure equalization tube?
- How does the eardrum behave under pathological conditions such as calcification or an excess of fluid in the tympanic cavity?
- How would activation of one or both muscles in the middle ear influence the configuration of the ossicular chain?
Finally, we developed a new method to measure the 3D motion of the middle ear ossicles with an accuracy of about 10 µm. The method combines the X-ray stereoscopy with the grayscale information obtained from X-ray shadow images. Moreover, the setup makes use of a single X-ray point source, unlike the classic stereoscopy setups with two sources, and does not require the structures to be visually exposed.

Method

Morphology of soft and bony tissue of the human middle ear

In total six 3D models of the middle ear were constructed from microCT-scans of healthy human temporal bones. First, the middle ear was dissected from the temporal bone, and a small hole was drilled in the cavity side to enable the injection of staining fluid. To preserve the samples, they were stored for 4 days in a 4% formaldehyde solution. Afterwards, they were stained for 5 days in a 3% solution of phosphotungstic acid (PTA) in water (Metscher 2009a,b).

After flushing the samples with water to remove the residual PTA, they were scanned with the X-ray micro-computed tomograph of the centre for X-ray micro-computed tomography at Ghent University (Masschaele, 2007). Resolutions ranging from 19 to 23 µm were obtained.

The semi-automatic segmentation and model triangulation was done in Amira 5.3 (Visage Imaging).

Finite-element modeling of the middle ear

Using the same software package, we can generate finite-element volume models, taking care to avoid intersections, holes, badly-oriented faces etc. Important is now to attribute the correct material parameters to all elements of each component. Next, using the open source package FEBio or the commercial COMSOL package, we perform simulations based on physical interactions between the finite number of elements (> 100,000) from which the models are built.

X-ray stereoscopy with grayscale analysis for dynamic middle ear behavior

Rabbit and gerbil temporal bones were dissected from euthanized animals. A small hole was drilled in the cavity, to mark the ossicles in the middle ear with tungsten beads with a mass that is negligible in comparison to the ossicles.

First, two X-ray images were recorded – initially with a SkyScan 1074 and later with the custom-made scanner from UGCT – from different directions by rotating the object over a 90 degree angle between imaging, while a sinusoidally changing pressure was applied to the middle ear. The exposure time of each X-ray image was identical and matched with (an integer multiple of) the period of motion. Since the ossicles moved during the X-ray imaging due to the applied air pressure, the integrated intensity of the moving beads is smeared out: different gray values are observed along the paths of motion of the marker points due to the motion speed. When a marker remains for a long time in a given position, more X-rays are absorbed and vice versa, as shown in figure 1 right.
Figure 1: Snapshots of X-ray shadow images show three tungsten beads on top of the malleus ossicle of a gerbil at:
(a) rest/static state before applying pressure and
(b) during linear loading of the tympanic membrane with a pressure of 2 kPa at a frequency of 50 Hz.
The scale bar is 500 µm.

Using the disparity between the two X-ray stereograms, we can calculate the 3D coordinates of each marker at its outermost positions of the periodical motion in the World Coordinates System (WCS), from the respective marker positions in both images in Camera Coordinates System (CCS) (Salih et al. 2011a,b). We thus obtain the 3D coordinates of the path of motion.

Next, assuming a linear path, time information (and thus speed) at each point along the path of motion can be obtained by integration of the gray scale values (Salih et al. 2011b).

Results

Morphology of soft and bony tissue of the human middle ear

Results are shown here for one temporal bone scan, although six scans were made in total. Figures 2(a) and 2(b) both show a cross section through the middle ear. Soft tissue structures such as the tympanic membrane and its connection with the manubrium of the malleus (manubrial fold), the incudomallear joint, and the middle ear muscles can clearly be seen on the images. Particularly the fibrous structure of both tensor tympani and stapedius muscle is nicely shown. Also visible are mucosal layers and the chorda tympani.

Figures 3(a) and 3(b) show 3D VRT (Volume Rendering Technique) images of the ossicles and the eardrum. Surrounding structures were removed by cropping the image dataset around the ossicles or eardrum respectively. The ossicles are colored from segmentation data, and the soft tissue is visualized by choosing the appropriate threshold. The simple threshold selection demonstrates how easily the soft tissue can be distinguished from the background. After segmentation (outline the separate structures in each section) we can generate a surface model.
Figure 2: Cross sections through the middle ear: (a) through incus and malleus, and (b) along the tensor tympani muscle.

Figure 3: 3D VRT images of (a) the ossicles, and (b) the tympanic membrane.

Finite-element modeling of the middle ear

We succeeded in creating a realistic finite-element volume module from the segmented microCT data surface model: Figure 4 shows the 3D surface model that was constructed from the microCT image data. The model contains unprecedented data and insights on the following structures:

- malleus, incus, and stapes
- tympanic membrane, and connection with manubrium of malleus
- incudomallear joint, incudostapedial joint, and annular stapedial ligament
- posterior incudal ligament, anterior mallear ligament
- chordi tympani nerve, tensor tympani and stapedius muscles and tendon

Simulations are on-going but results are still preliminary. Hence, we show the state-of-the-art at the conference.
X-ray stereoscopy with grayscale analysis for dynamic middle ear behavior

With the new method that we developed, the 3D displacement of the middle ear ossicles in both gerbils and rabbits has been studied as a function of quasi-static pressure changes (≤ 50 Hz). Using the time information obtained from the grayscale values with the stereoscopy method, the motion of the ossicles can be reconstructed and demonstrated on the high-resolution fully-scanned microCT computer model of the middle ear, shown in figure 5 (Salih et al. 2012).

A study on a controlled phantom object showed that the 3D displacement can be calculated with an accuracy of approximately 10 µm while the velocity along the path of motion can be reconstructed with a RMSE of 5%.

Conclusion

Through a soft tissue staining method, we were able to generate the highest resolution and most complete (human) middle ear model from microCT to date. The resulting 3D models give new insights in the morphology and biomechanics of the middle ear. Furthermore, we developed a new X-ray stereoscopy method, which through grayscale analysis, is able to measure periodic motion of the ossicular chain in the middle ear.
Figure 6: Snapshot 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) malleus, (b) the incus, (c) the stapes and (d) the cochlea. The marker points or beads on the ossicles are presented in the model as well (in red). For the malleus (a): green represents the +1 kPa position, blue its rest position at 0 kPa, and orange/pink the -1 kPa position. For the stapes (c): green represents the +1 kPa position, purple its rest position at 0 kPa, and transparent orange/pink the -1 kPa position.

Acknowledgements
This work was supported by the Flemish agency for Innovation by Science and Technology (IWT), the Research Foundation – Flanders (FWO), and the Sudanese Alneelian University. We would like to thank Dr. Jean-Marc Gerard of the university hospital St. Luc and Joris Walraevens of Cochlear technology centre Belgium for putting temporal bones at our disposal. Special thanks go to UGCT, Manuel Dierick and Pieter Vanderniepen for the use of their scanning facilities.

References


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