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CONTRAST-ENHANCED MICRO-COMPUTED TOMOGRAPHY OF THE HUMAN MIDDLE EAR

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INTRODUCTION

The middle ear acts as an impedance matching transformer to efficiently transfer sound from low-impedance air in the ear canal to high-impedance cochlear fluids. The middle ear includes the tympanic membrane (TM) and three ossicles (malleus, incus and stapes) which are supported by ligaments and muscles as shown in Figure 1.

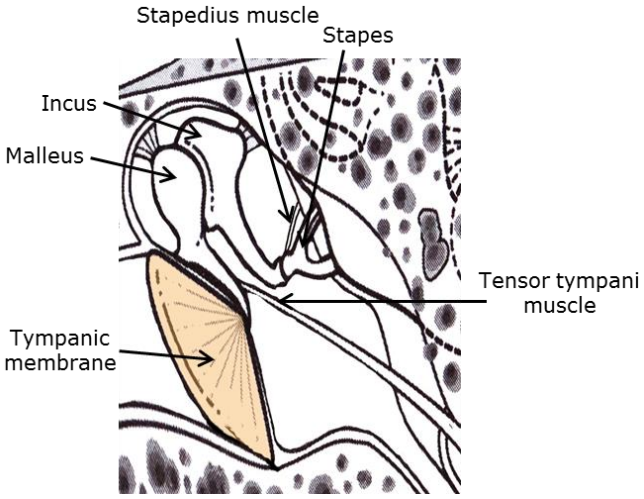

Figure 1 - Anatomy of the human middle ear. Modified from Descouens (1).

Starting with the pioneering work of Funnell and Laszlo (1978), numerous finite-element models (FEMs) have been developed to quantify the function of the middle ear. High-resolution imaging of middle-ear geometry is essential for the development of accurate FEMs. Micro-computed tomography (μCT) is widely used because of its ability to image the bony structures of the middle ear. However, in μCT images, it is difficult to visualize soft tissues such as the TM, tensor tympani and stapedius muscle because of low contrast.

One method to improve the contrast of μCT images is to use any one of several chemical compounds such as osmium tetroxide, phosphotungstic acid (PTA) or iodine potassium iodide (IKI) (2). Aerts et al used PTA to enhance the contrast of soft tissues when acquiring μCT images of the middle ear (3). To the best knowledge of the authors, there is no published work evaluating the efficacy of staining middle-ear soft tissues with IKI.

Therefore, the objective of this study is to evaluate IKI solution as a contrast agent for μCT imaging of middle-ear soft tissues.

MATERIALS AND METHODS

Two freshly frozen temporal bones were used in this project. Ethics approval was received from the Chief Coroner of Ontario. Samples were first fixed in a 4FIG (3.7% formaldehyde + 1% gluteraldehyde in phosphate buffer) bath for 5 days. Furthermore, one sample was stained using 1% IKI solution for 2 days. Then, the bones were dehydrated using an ethanol series.

The samples were imaged using the eXplore Locus μCT scanner (GE Healthcare Biosciences, London, Ontario, Canada). The scanner operated with an x-ray tube voltage of 80 kV and a current of 0.45 mA. Using a modified conebeam algorithm (4), the data were reconstructed into a 3D image volume at an isotropic voxel size of 20 μm.

In the current work we focused on contrast improvement of three soft tissues, the TM, tensor tympani and stapedius muscle. After obtaining the μCT dataset, 11 equally-spaced slices were selected for each tissue to calculate the contrast-to-noise ratio (CNR) using the formula:

$$CNR = \frac{\bar{x}_{tissue} - \bar{x}_{air}}{STD_{air}} \quad \text{Eq. 1}$$

where $\bar{x}$ is the mean tissue or air intensity and STD is the standard deviation (5). The intensities of each tissue were sampled along a line drawn through that tissue. Also, the air region is selected as a square of 30x30 voxels close to the TM.

RESULTS

Two human temporal bones were imaged with μCT, resulting in two isometric data stacks of 774 slices each. To illustrate differences in contrast between the unstained and stained images, two sample slices through each dataset are shown in Figure 2. The tympanic membrane is easier to visualize in the stained image (Figure 2b) but harder to notice in the unstained image (Figure 2a).

Mean image intensities and variations in them are shown in Table 1.

Table 1 – Average measurements of air, TM, tensor tympani and stapedius muscle intensities in Hounsfield Units (HU)

	Unstained	Stained
Air	-359±144	-446±179
Tympanic membrane	96±243	502±402
Tensor tympani	384±145	917±208
Stapedius muscle	453±181	473±251

Corresponding CNRs computed using the data in Table 1 and Eq. 1 are given in Table 2. CNRs of the TM and tensor tympani were higher in the stained image compared to that in the unstained image, while the CNR of the stapedius muscle did not change significantly.

Table 2 - CNR of TM, tensor tympani and stapedius muscle

	Unstained	Stained
Tympanic membrane	3.2	5.3
Tensor tympani	5.1	7.6
Stapedius muscle	5.7	5.1

To visualize the differences in intensity distributions, for each tissue type, intensity histograms were calculated and fitted with Gaussian distributions. Histograms of air, TM and tensor tympani for both samples are given in Figure 3.

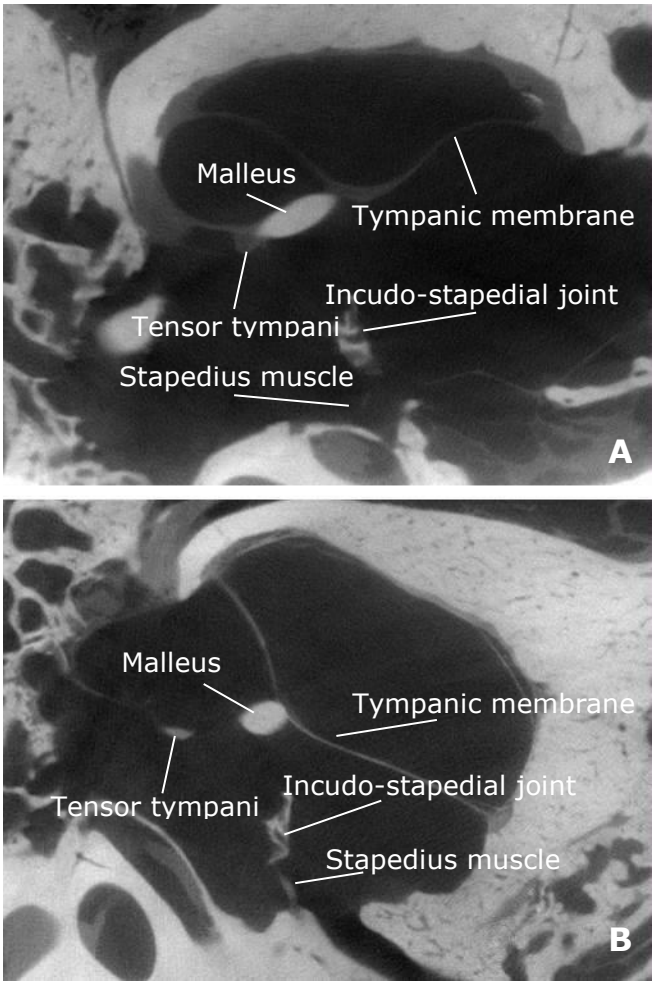

Figure 2 – μCT slices of unstained and stained images. A, unstained sample, B, stained sample.

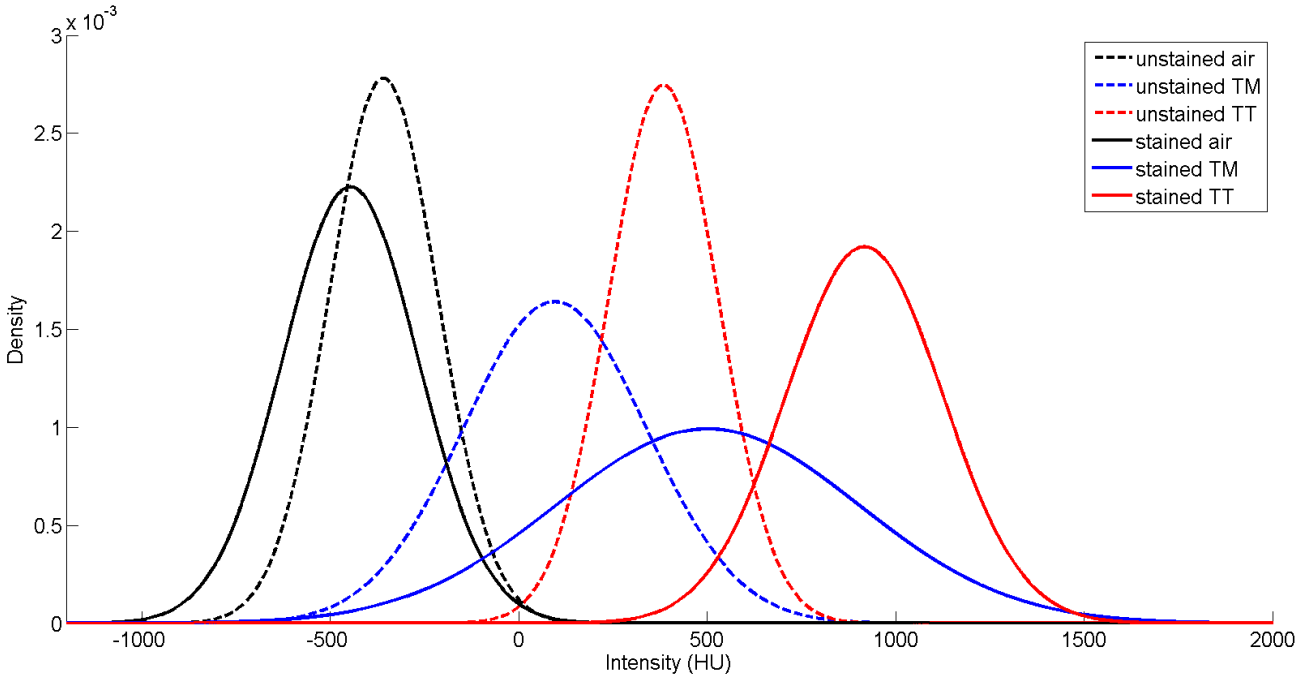

Figure 3 - Histograms of air, TM and tensor tympani (TT) in stained and unstained samples

DISCUSSION

Osmium tetroxide has been the common choice for μCT scanning, but it is toxic, expensive, difficult to dispose of and has low penetration rate (6). IKI and PTA have similar contrast-improving capabilities (6), but IKI is preferred in this study because it will not potentially erode the bones or soft tissues.

After staining, the CNRs of the TM and tensor tympani were increased by 66% and 46%, respectively, while no significant change in CNR was measured for the stapedius muscle. The lack of significant change for the stapedius muscle could be because of its smaller size which results in less contact area with the IKI solution which could affect the absorption of IKI. In addition, we observed a general increase of standard deviation of x-ray intensity in soft tissues in the stained image. This could indicate variability of IKI binding.

There are other alternative methods to obtaining high quality images of the middle ear. The main alternatives are obtaining geometry using histological sectioning and using combined imaging modalities such as μCT and OPFOS (orthogonal-plane fluorescence optical sectioning) (7). Although histological sectioning has better resolution than scanning (as low as 1 μm), there are technical challenges in sample preparation. On the other hand, OPFOS requires extensive chemical processing to render all tissues transparent because it is an optical technique. For these reasons, contrast-enhanced μCT is less labour intensive and preferred in this study.

CONCLUSION

Staining the middle ear with IKI solution improves the contrast of soft tissues such as the TM and tensor tympani when imaging using μCT.

Future studies will focus on optimization of contrast agent concentration, staining time and scanning protocol.

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REFERENCES

[1] D. Descouens, "Eardrum schema," Wikicommons.com, 7 November 2009, Web, accessed 10 February 2014.

[2] B.D. Metscher, "MicroCT for developmental biology: A versatile tool for high-contrast 3D imaging at histological resolutions," *Developmental Dynamics*, vol. 238(3), pp. 632-40, 2009.

[3] J. Aerts, J. Dirckx, M. Dierick, L. Van Hoorebeke, "High-resolution 3D surface model of bone and soft tissue structures in the human middle ear" *ARO mid-winter meeting*; San Diego, California, USA, 2012.

[4] L. A. Feldkamp, L. C. Davis, J. W. Kress. "Practical cone-beam algorithm," *Journal of the Optical Society of America A.* vol. 1(6), pp. 612-9, 1984.

[5] J.T. Bushberg, *The essential physics of medical imaging*, 3rd ed. Philadelphia: Wolters Kluwer Health/Lippincott Williams & Wilkins, 2012.

[6] B.D. Metscher, "MicroCT for comparative morphology: Simple staining methods allow high-contrast 3D imaging of diverse non-mineralized animal tissues," *BMC physiology*, vol. 9(11), 2009.

[7] J.A. Buytaert, W.H. Salih, M. Dierick, P. Jacobs, JJ. Dirckx, "Realistic 3D computer model of the gerbil middle ear, featuring accurate morphology of bone and soft tissue structures," *Journal of the Association for Research in Otolaryngology*, vol. 12(6), pp. 681-96, 2011