

Abstracts – MEMRO 2006, Zurich July 27–30, 2006

4th International Symposium on Middle Ear Mechanics in Research and Otology

P9

High resolution 3-D imaging and modelling of middle ear ossicles and soft tissue structures of intact Gerbil temporal bones, using orthogonal-plane fluorescence virtual sectioning

J. Buytaert, J. Dirckx, Antwerp; Belgium

Detailed models of middle ear morphology are an important input to improve realism of computer models of middle ear mechanics. Histological sections are used to produce high quality models, but the method is extremely work intensive. CT data deliver models of the bony structures with reasonably good resolution, but soft tissue is much more difficult to image. Orthogonal-plane fluorescence optical sectioning or OPFOS, is an important additional technique which, after adequate specimen preparation, produces high quality, perfectly aligned sectional images in nearly real time, of both bone and soft tissue. The OPFOS technique was introduced by A.H. Voie (J. Microsc. 170, 1993), and was used to construct models of cochlea, with a slicing resolution of about 14 μ m. We have improved the OPFOS technique, so that slice thicknesses of less than 3 μ m can be achieved to measure shape of ossicles, muscles, tendon and even blood vessels in the middle ear. In conventional OPFOS, a plane of laser light is projected through a fluorescent specimen, and the light emitted from that plane in the object is observed in orthogonal direction, thus showing in real time a virtual section. To apply the technique, specimens need to be completely transparent, refractive index matched to a surrounding fluid, and made fluorescent. This is achieved by decalcifying, dehydrating, clearing with Spalteholz fluid, and finally staining the specimen. The thickness of the laser plane is always a trade-off with the image size. Using improved optical design and adding motorized scanning along a direction of the image plane, we were able to overcome this limitation and reduce slicing thickness to 2 μ m, with an in-plane resolution of 1.5 μ m. To establish objective measurements of resolution, we compared our OPFOS results to data obtained from SEM, on a custom made plastic test object. We will show data of middle ear ossicles and soft tissue structures, such as the stapedial muscle and tendon. Measurements were obtained in preparations of intact temporal bones of Gerbils, as the technique allows region-of-interest scanning within a larger specimen. From the section data, we reconstructed 3-D models of the middle ear. In these models we are also able to show for instance the stapedial artery, which is nearly invisible on CT. Sections from our high resolution data show the hollow structure of the stapes head, cavities within the body of the ossicles, and other functional details. Fusion with CT data, obtained on the same specimens, can be used to discriminate between bony structures and soft tissues.