Conclusions and discussion
Conclusions and discussion

With regard to the objectives postulated in the general introduction;

Objective 1. Evolution of inner ear morphology
Taxonomically speaking vertebrates are descendents of Craniata (animals with a skull). The investigated species, fish, reptile, amphibian, bird and mammal are not just descendents in this order. Amphibians and amniotes (tetrapods with a terrestrially adapted egg) originate from fish. Amniotes can be divided into mammals and reptiles, whereas birds originate from crocodiles.
Remarkable is the evolvement of the auditory part of the labyrinth. From a small lagena in fish to a distinct lagena and cochlear duct in lizards and birds. The vestibular part of the labyrinth is a very stable feature in the described development. The ultrastructure of both the auditory and the vestibular system also appears to be stable factor. The lateral line organ in fish and some amphibians is an organ with the same ultrastructural components as the inner ear, but it is questionable if it is the precursor of the inner ear. The endolymphatic sac seems a feature of importance, because it is present in most species (except from primitive fish).

Objective 2. Three dimensional reconstruction
The spatial resolution of OPFOS-microscopy is about 20 µm (Voie, 2002). A better resolution can be reached with light microscopy. When a resolution of 20 µm is sufficient, OPFOS microscopy is a superior technique for 3D-reconstruction of inner ear structures in animals.
OPFOS-microscopy is especially useful in depicting specimens containing bone, fluids and membranes. Small bony structures are easy to decalcify. For bigger bony structures the decalcification step in the described method must be prolonged. Soft tissue like fat, muscle and neural tissue can not be made transparent and thus can not be transluminated. Even after careful dissection, unremovable tissue forms shadows in the image.
The whole-specimen character of the OPFOS-method makes it very suitable for 3D-reconstruction. The constituting 2D-images do not need to be "manually" rotated or stretched in order to achieve the best alignment for the 3D-reconstruction of the specimen. Furthermore the whole-specimen character of the method results in absence of possible distortions due to blade-passages of the microtome, as can be the case in for instance light microscopy.
The specimen can be suspended from a wire in the specimen-chamber in various positions and it can be illuminated from different angles by the laser beam.
The prepared specimen can be used over and over again with a time limit of a few months. (Due to photo bleaching the fluorescence of the specimen will gradually decrease).
Objective 3. Insight in morphology and function inner ear

Three dimensional reconstructions of the area of the round window membrane and the cochlear aqueduct of the guinea pig show that the membrane has a pouch-like extension. The tip of the membrane extension is adjacent to the opening of the cochlear aqueduct. The supposed function of this extension is a role in regulating flow resistance in the cochlear aqueduct as a function of inner ear fluid pressure.

Three dimensional reconstruction of the utriculo-endolymphatic valve in guinea pig shows a rigid ‘arch-like’ configuration of its lip and a duct with the shape of a flattened funnel. In the direction of the endolymphatic duct and sac this funnel runs into a very narrow duct. The side opposing the valve lip has a thickness of only one cell-layer, and is as result highly compliant. It is most likely that opening or closure of the valve occurs through movement of the flexible base away from or toward the relatively rigid valve lip and not by movement of the lip itself.

The described hydrodynamic model about the functioning of the endolymphatic sinus displays that an increase in perilymphatic pressure compresses the sinus. The resulting pressure increase inside the saccule will precisely counteract the pressure that tries to compress the sinus.

Three dimensional reconstruction of the area between the utricle and saccule in pigeon shows a valve-like structure. Its appearance equals the appearance as described for guinea pigs. At the utricular side the valve is slit-shaped. The ridge overhanging the slit appears as a relatively rigid structure.

When reconstructing the pigeon inner ear by means of OPFOS-microscopy the shape of the semicircular ducts appeared to be far from semicircular. The ducts are not lying in a one flat plane, especially not the anterior and the lateral canal.

The cochlear duct in pigeon was precisely reconstructed based on light microscopical slides. Volumes of endolymphatic space, i.e. saccus endolymphaticus, cochlear duct, saccule and the entire endolymphatic space, are given.

General discussion

In search for a whole-specimen imaging technique to reconstruct the inner ear in three dimensions, Orthogonal-Plane Fluorescence Optical Sectioning (OPFOS; Voie et al. (1993)) appeared very useful. Due to soft tissue and bone characteristics in different animals OPFOS-microscopy also showed its imperfection. When accurately processed, light microscopy can be a useful addition in three dimensional reconstruction.

OPFOS-microscopy is a very valuable tool in postmortem 3D-reconstruction of the inner ear in animals. In future work we are aiming for reconstruction of the human inner ear. The preparation of such a specimen will be much more time-consuming since the decalcifying process will take much more time. The specimen itself must be dissected very carefully. The excess of
temporal bone has to be drilled away to minimalize the bone diameter protecting the membranous inner ear. This will optimize the thickness of the tissue the LASER-beam has to pass through. The ultimate goal will be the reconstruction of the inner ear of a patient with Menière’s disease. Hopefully it will confirm the presence of a hydrops in the endolymphatic system as shown in a light-microscopical slide in chapter 1.

Buytaert and Dirckx (2007, 2009) have improved OPFOS-microscopy with their High Resolution (HR)OPFOS-technique by strongly reducing slicing thickness, resulting in improvement of resolution by about five times.

Three dimensional reconstruction of the round window membrane and the cochlear aqueduct shows a pouch-like extension. The aqueduct is obstructed at the middle ear side when the inner ear fluid pressure is low and the membrane is moving inwards. The loose connective tissue inside the aqueduct is stretched when the round window membrane moves outward, resulting in an open aqueduct due to low flow resistance (Wit et al., 2003). These results sustain the findings that the resistance for fluid flow through the aqueduct is dependent on the position of the round window membrane (Feyen et al., 2004).

The exact function of Bast’s valve remains uncertain. We believe, as do Bast (1937) and Schuknecht and Belal (1975), that a high perilymphatic pressure could close the valve by moving the opposing wall against the valve lip. The rigid structure of this lip (which is actually more an arch than a lip) suggests that the normal situation is an open entrance of the utricular duct. On the other hand if the normal situation is a closed valve, one could speculate that the true function of the valve is the maintenance of endolymphatic pressure in case of a catastrophe. For instance in superior canal dehiscence syndrome of perilymphatic leakage after high pressure trauma, the perilymphatic pressure will rapidly fall. As a consequence Bast’s valve will be opened. The endolymph will be redistributed to maintain function in both the vestibular as well as the auditory part of the labyrinth. In unpublished work on the Lizard inner ear, an utriculo-endolymphatic valve-like structure can be distinguished at the utricular side of the duct that connects utricle and saccule. It is reasonable to assume that the presence of a valve at exactly that location has a relation with the presence of a separate hearing organ.

The main obstacle in the dispute about presumed function of the endolymphatic sinus is the absence of a possibility for outflow of endolymph in our hydrodynamic model. The inner ear is a closed, dense (bony labyrinth) entity, containing two closed fluid compartments (endolymphatic and perilymphatic space). Perilymph will disappear through the cochlear aqueduct when perilymphatic pressure exceeds the CSF-pressure. But the change of pressure exerted by perilymph on the endolymphatic compartment
is equally distributed over the whole endolymphatic compartment and thus will not give an opening or closure of the endolymphatic sinus.

By means of the combination of 3D-reconstruction based on orthogonal-plane fluorescence optical sectioning (OPFOS) microscopy (Voie et al., 1993) as well as conventional light microscopy it is possible to reconstruct the whole pigeon inner ear. To our knowledge the presented figures are the first three dimensional reconstructions of the pigeon inner ear.

The investigations for this thesis were started with the idea that studying the evolution of the inner ear, and in particular the evolution of its endolymphatic system, would yield insight in the importance of the endolymphatic system for inner ear homeostasis. For this purpose microscopic studies of the inner ears of various species were undertaken. The primary expectations of a possible wider insight in the pathogenesis of Ménière’s disease, have failed. This thesis can also not offer these answers after almost a century of fundamental and clinical research. Structures like Bast’s valve, the entrance of the cochlear aqueduct and the endolymphatic sinus were reviewed for their morphology and (possible) function. This resulted in 3D-reconstructions of these specific parts and of entire endolymphatic systems.

The investigations and literature reviews for this thesis demonstrated new information about the inner ear endolymphatic system. That the endolymphatic system is important for inner ear functioning can be concluded from the fact that it has not essentially changed during evolution, apart from some minor details.
References

Bast T.H., 1937. The utriculo-endolymphatic valve and duct and its relation to the endolymphatic and saccular ducts in man and guinea pig. Anat Rec 68, 75-93


Wit H.P., Feijen R.A., Albers F.W., 2003. Cochlear aqueduct flow resistance is not constant during evoked inner ear pressure change in the guinea pig. Hear Res 175, 190-199