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# Research paper

# The creation of geometric three-dimensional models of the inner ear based on micro computer tomography data

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1. Introduction

# ABSTRACT

The modeling of the mechanical process of hearing requires an accurate geometrical model of the inner ear (cochlea). The purpose of this study was the creation of a 3-D model of the fluid chambers of Guinea pig cochlea, which could serve as a basis for further mechanical modeling. Micro computer tomography used in this study is a noninvasive method to visualize bony structures. The visualization of the membranous labyrinth was achieved by additional staining of the specimen with OsO<sub>4</sub>. The resulting stack of images has been transformed into a cylindrical coordinate system. To suppress noise on tomography images, a nonlinear smoothing method, anisotropic diffusion, were applied. A new approach has been proposed to estimate algorithm parameters automatically. Then, a segmentation using active contours (snakes) was performed. In this study, a new energy linking the contours on adjacent slices has been added to the standard approach. This compensates the inconsistencies between adjacent contours. The images segmented in this way were used as a basis for a 3-D reconstruction of the hearing organ.

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The process of sound perception in the inner ear is a complex interaction between fluids and solid structures in snail-shaped canals (scalae) called the cochlea. The mechanical and fluid mechanical modeling of these processes requires an accurate geometrical model of the cochlea as a basis. Such a model can be used for the understanding of fundamental physiological processes of hearing as well as for applied medical tasks. These aims require the modeling of all structures of the hearing organ, including bones and soft tissue structures (tectorial membrane, Reissner's membrane, organ of corti). Previous studies have shown that the size and the shape of these structures change along the cochlea (Fernández, 1952; Lim, 1980; Raphael and Altschuler, 2003). These morphological changes are essential for the discrimination of frequency in the organ of hearing. The mapping of these changes in the form of a 3-D model of the cochlear scalae and membranes is an important step to provide reliable geometrical data of the entire organ and model its tonotopical mechanics.

There are a number of methods available to study the structure of the inner ear. The method most frequently used is the histological slicing of the cochlea with subsequent light microscopic observation and scanning of the slices. This approach is capable of visualizing membranes as well as bones, and has been mainly used to estimate discrete geometrical data of the cochlea, i.e., the positions of selected features or points (Kawano et al., 1996; Sato et al., 1999; Shinomori et al., 2001; Jin et al., 2006). Mechanical sectioning of the inner ear unavoidably leads to distortions, especially of the membranes. A reconstruction based on this method usually does not deliver sufficiently precise models of the structures. In particular, the coupling of neighboured slices has not been realized in practice with required smoothness (Harada et al., 1990; Tinz, 2005; Li et al., 2006).

An entirely different approach is used for the imaging of the cochlea with magnet resonance-tomography (MRT) and computer

Abbreviations: 2-D, two-dimensional; 2.5-D, two-and-a-half-dimensional; 3-D, three-dimensional; CAD, computer-aided design; CT, computer tomography; DFG, Deutsche Forschungsgemeinschaft (German research foundation); micro-CT, micro-scopic computer tomography; MRT, magnet resonance tomography; TEM, transmission electron microscopy; TIFF, tagged image file format; VRML, virtual reality modeling language

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tomography (CT). These methods are capable of non-invasively delivering data that can be transformed into a 3-D model by image processing/segmentation algorithms.

MRT is often used to create soft tissue models of the cochlea, particularly *in vivo*. The main disadvantage of this technique lies in the difficulty or even inability to display bony structures. Nevertheless, its main application has concentrated on the reconstruction of the fluid chambers of the inner ear (Thorne et al., 1999; Counter et al., 2000).

CT, on the other hand, is primarily used for the imaging of bony structures. It is also used for procuring geometrical data of the cochlea in certain important points (Spoor and Zonneveld, 1998; Yoo et al., 2000; Christensen et al., 1999). A variation of CT is microscopic computer tomography (micro-CT) with a resolution of about  $1-20 \mu$ m. Attempts to model the ear with micro-CT in the past have shown that this method is sensitive enough for a detailed investigation of the cochlea; however, there exist problems with soft tissue imaging, especially with fine cochlear membranes (Vogel, 1999; Lane et al., 2004; Uzun et al., 2007).

All imaging methods based on physical radiation such as X-rays, light, sound, particle beams or magnetic resonance produce artifacts (noise) that are displayed on the images together with the real object. Often, this noise has a high characteristic frequency and makes the discrimination of finer structures difficult. It has the form of small stochastically ordered intensity differences from the background and dimensions ranging from two to, maximally, three pixels. The general-purpose 3-D modeling software cannot adequately differentiate between finer soft tissues and the noise. Regardless of contrasted visualization of structures, the intensity of soft tissue is not sufficient to segment requested features with an intensity threshold.

The standard method of improving quality is to denoise images before segmentation. Linear filtering is the simplest way of image denoising. A linear filter is the convolution of an image with a kernel function. Low pass filtering, which filters out high frequency noise, can be applied by a convolution with a 2-D Gaussian kernel. It is a simple approach, but besides the noise, it also tends to erase fine structures. In our case, fine structures (Reissner's membrane) are the essential part of the image, which must be preserved. The more complex edge-preserving nonlinear algorithms with properly tuned parameters may perform better in such conditions. The anisotropic diffusion algorithm is a possible candidate for nonlinear smoothing of images before segmentation. This algorithm was developed in 1990 (Perona and Malik, 1990). It preserves the membranes from being erased due to some kind of non-locality of smoothing. The boundary between regions could be partially invisible, but the residual visible part slows down the diffusion process.

Nevertheless, there is still a loss of finer structures, due to the weakness of the initial signal from thin and delicate membranes and/or insufficient staining of the structures. It also manifests itself in structural discontinuity. This places significant demand on the reconstruction of some of the finer structures. For such cases, active contours, also called snakes, have been developed in 1988 (Kass et al., 1988). They have a broad use (Wong et al., 1998; Ji and Yan, 2002; Ladret et al., 1999), including application in biological imaging (Kang, 1999; Valverde et al., 2004). The snake algorithm reconstructs the lost (invisible) part of object boundaries, so that the reconstructed images can be used as an input for the 3-D modeling software.

An application of a similar approach using constrained snakes was presented to segment cells in volumetric histo-pathological images (Umesh Adiga and Chaudhuri, 1999). The problem of this approach lies in the necessity of manual drawing of initial contours of the snake. In the case of a 3-D reconstruction, this technique would require manually drawing several hundreds of contours. The objective of this study was to develop a semi-automatic method for the creation of 3-D models of the cochlea on the basis of micro-CT data. It introduces the following elements: anisotropic diffusion, the projection of the cochlea into a cylindrical coordinate system and the implementation of a 2.5-D active contour algorithm.

# 2. Materials and methods

# 2.1. Experimental part

# 2.1.1. Animals

A healthy pigmented guinea pig weighing 300 g (Charles River, Germany) was anesthetized in a CO<sub>2</sub> atmosphere and decapitated. The auditory bullae were removed and placed in an artificial perilymph (HEPES-Hanks solution (invitrogen), 300 mOsm, pH 7.34). The cochleae were drilled out of the surrounding bone structures. The semicircular ducts and the ossicular chain were removed. The intact round window membrane was pierced with a fine injection needle and the tympanic and the vestibular scalae were slowly perfused with a neutrally buffered 4% formaldehyde solution (SAV Liquid Production) containing 2 mM CaCl<sub>2</sub> (Sigma). Then, the specimen was placed into an equally composed solution for 24 h. After fixation, the staining of the soft tissues was achieved by a slow perfusion of scalae with a 2% OsO<sub>4</sub> (Serva) in 0.1 M sodium cacodylate buffer and subsequent dipping of the specimen into the same solution for 2 h. The specimen was then rinsed in a phosphate buffer solution and immersed into polyethylene glycol 400 (Merck). The conservation in polyethylene glycol is based on the replacement of water by this substance, thereby preserving the specimen from drying out. The process took two weeks to complete. Then, the remaining polyethylene glycol was removed. This completes the preparation and the specimens are ready for recording (Fig. 1).

#### 2.1.2. Micro-CT system

The images were taken with a micro-CT device (Fraunhofer ERZT) with a microfocal X-ray source by Feinfocus GmbH. It has a focus of ca. 1  $\mu$ m and an X-ray energy spectrum from 10 to 225 keV. The beam is controlled by a seven-axes precision manipulator (Agfa NDT Pantak Seifert GmbH and Co. KG). The system allows a maximum resolution of 2  $\mu$ m. However, due to the big size of the specimen (ca. 6 mm), the highest possible resolution was limited to 10  $\mu$ m to keep the entire specimen in the field of vision of the tomograph. To achieve a present resolution of 4  $\mu$ m, it was necessary to record the object in smaller parts and subsequently concatenate those parts to a single file.

The specimen was put onto a rotation plate and radiographed during a step-by-step 360° rotation. It delivered 800 projections in different orientations. These were transmitted to a computer cluster consisting of 8 PCs. This allowed the simultaneous observation and reconstruction of the object's volume model. The resulting data buffer could be transformed in every 3-D orientation. It provided the possibility to show and measure surfaces and inner structures. The density of tissues was represented by values on the greyscale. For documentation, the resulting data was saved to a stack of TIFF images. A file of this type could be further used to create various 3-D geometry and CAD models using commercially available software.

# 2.2. Image processing and 3-D reconstruction

For the purposes of image processing, our own program "IPTools" has been used. This is a freeware program downloadable at http://www.tu-dresden.de/medkhno/sites/wissenschaft/mitte-lohr/innenohr/innenohr\_en.html#iptools.





Fig. 2. Cylindrical coordinate transformation.

**Fig. 1.** Cochlea specimen prepared for micro-CT. The line denotes the approximate position of the longitudinal axis of the cochlea.

# 2.2.1. Cylindrical projection

The CT stack was transformed into a cylindrical coordinate system as presented in Fig. 2. The longitudinal axis (Fig. 1) was estimated manually from the image and defined by two points ( $x_0$ ;  $y_0$ ;  $z_0$ ) and ( $x_1$ ;  $y_1$ ;  $z_1$ ) with the vector difference ( $\Delta x$ ;  $\Delta y$ ;  $\Delta z$ ). The Cartesian coordinates x, y and z are related to the cylindrical r,  $\varphi$  and  $z_c$  as

$$\begin{pmatrix} x \\ y \\ z \end{pmatrix} = \begin{pmatrix} x_0 \\ y_0 \\ z_0 \end{pmatrix} + \frac{z_c}{S_1} \begin{pmatrix} \Delta x \\ \Delta y \\ \Delta z \end{pmatrix} + \frac{r}{S_1 \cdot S_2} \begin{pmatrix} \Delta y \cdot S_1 \cos \varphi - \Delta x \, \Delta z \sin \varphi \\ -\Delta x \cdot S_1 \cos \varphi + \Delta y \, \Delta z \sin \varphi \\ S_2^2 \sin \varphi \end{pmatrix}$$
(1)

with

$$S_1 = \sqrt{\Delta x^2 + \Delta y^2 + \Delta z^2} \tag{2}$$

and

$$S_2 = \sqrt{\Delta x^2 + \Delta y^2} \tag{3}$$

Since pixels of the transformed coordinate system do not match the pixels of the initial system, it is necessary to interpolate the intensities of the pixels of the transformed system. Here, tri-linear interpolation has been used. This method interpolates the intensity of a new pixel linearly from the intensities of the eight nearest pixels in the initial system (Fig. 3).

It follows the tri-linear polynomial:

$$\tilde{I} = ax'y'z' + bx'y' + cy'z' + dx'z' + ex' + fy' + gz' + h$$
(4)

where *I*~ is the interpolated intensity and *x*', *y*', *z*' are local coordinates with values between 0 and 1. For every *P* of the new coordi-



nate system, this polynome is thereby defined by eight edge conditions. This gives a completely defined system of eight equations. The solution of this equation gave the parameters a-h, which were then inserted into the equation together with the local position of P in the respective neighbourhood cube. These parameters are presented in the Appendix. Since the equation was solved for local coordinates, its solution could be applied to every P.

#### 2.2.2. Nonlinear filtration using anisotropic diffusion

Nonlinear filtering was performed with anisotropic diffusion. The basis of a diffusion algorithm is presented by the common physical diffusion formula:

$$\frac{\partial C}{\partial t} = \nabla \cdot (D\nabla C) \tag{5}$$

Here, the physical meaning of the substance concentration C is replaced by the greyscale intensity I, D is the diffusion coefficient and t is the time. In the case of the anisotropic diffusion algorithm, it is defined by the following expression:

$$D = \frac{A}{1 + \left(|\nabla I| \cdot \lambda\right)^n} \tag{6}$$

where *A*,  $\lambda$  and *n* are algorithm parameters. The original approach sets *n* = 2 (Perona and Malik, 1990). In this study, *n* = 4 yielded better performance. Thus, (5) becomes for the 2-dimensional case of an image:

$$\frac{\partial I(x,y)}{\partial t} = \nabla \cdot (D(x,y) \cdot \nabla I(x,y))$$
(7)

Smoothing was performed by diffusion of intensity during finite time. The time evolution of intensity was implemented by direct Euler's scheme:

$$I_{i+1}(x,y) = I_i(x,y) + \nabla \cdot (D(x,y) \cdot \nabla I(x,y)) \Delta t$$
(8)

where *D* was recalculated once every three time steps to avoid or reduce instability effects of the direct scheme.

Since the intensity distribution on an image is a discrete field of values, the differentials of intensities could be calculated by finite differences. In this study, we used symmetric non-smoothing approximation matrices. Their convolution with the image returns the approximated gradient vector or the Laplacian, respectively.

$$L_{\frac{\partial}{\partial x}} = \frac{1}{2} \cdot \begin{bmatrix} 0 & 0 & 0 \\ -1 & 0 & 1 \\ 0 & 0 & 0 \end{bmatrix}$$
(9)

$$L_{\frac{\partial}{\partial y}} = \frac{1}{2} \cdot \begin{bmatrix} 0 & 1 & 0 \\ 0 & 0 & 0 \\ 0 & -1 & 0 \end{bmatrix}$$
(10)

$$L_{\Delta I} = \frac{1}{2} \cdot \begin{bmatrix} 0 & 1 & 0 \\ 1 & -4 & 1 \\ 0 & 1 & 0 \end{bmatrix}$$
(11)

As the manual parameterization of the algorithm is time-consuming, the following algorithm was developed to estimate parameters and terminate the iteration automatically. Every time before the program computes a new pattern of diffusion coefficients D(x, y), it finds a maximum value of the intensity gradient. Then,  $\lambda$  was computed with the following formula:

$$\lambda = \frac{1.5}{|\nabla \hat{I}|} \cdot l \tag{12}$$

where  $|\nabla \hat{l}|$  is the maximum value of the intensity gradient on the current image, and l is a coefficient computed once from the first slice of the stack as follows:

$$l = \frac{|\nabla I_0|}{|\nabla I_{\text{def}}|} \tag{13}$$

where  $|\nabla \hat{I}_0|$  is the maximum value on the starting picture, and  $|\nabla \hat{I}_{def}|$  is the gradient at a user-defined border, which one wishes to preserve.

As the algorithm led to the sharpening of borders on the image, it caused a common increase of gradients. This would cause  $\lambda$  to decrease. The algorithm was stopped when  $\lambda$  fell to 75% of its initial value or after 75 iterations. The choice of parameter *A* is thereby not critical; in this study, it was *A* = 0.05.

In the case of the cochlea, it is likely that one wishes to track gradients not all over the picture, but in a smaller area defined by the user, which should include the membranes as the object of interest. Bones, having higher intensities than soft tissue structures, can disturb the enumeration of  $\lambda$ .

#### 2.2.3. Active contours

The standard snake energy balance, as proposed by Kass et al., 1988, was used to model the active contour:

$$E_{\rm tot} = E_{\rm cont} + E_{\rm curv} + E_{\rm ext} \tag{14}$$

where

$$E_{\rm cont} = \alpha \cdot \left| \nabla \nu(s) \right|^2 \tag{15}$$

$$E_{\rm curv} = \beta \cdot |\Delta \nu(s)|^2 \tag{16}$$

$$E_{\rm ext} = -\gamma \cdot \left|\nabla I\right|^2 \tag{17}$$

Here, v(s) is the curve function depending on the longitudinal coordinate *s*, and  $\alpha$ ,  $\beta$ , and  $\gamma$  are algorithm parameters.

The intensity gradient was computed as described above. The curve differentiations were adapted to the discrete space in cylindrical coordinates as follows:

$$\left|\nabla v(s)\right|^{2} = \frac{\left(r_{i} - r_{i-1}\right)^{2} + \left(z_{c,i} - z_{c,i-1}\right)^{2} + \left(r_{i} - r_{i+1}\right)^{2} + \left(z_{c,i} - z_{c,i+1}\right)^{2}}{2}$$
(18)

$$\left|\Delta\nu(s)\right|^{2} = \frac{1}{3} \cdot \left(\frac{1}{R_{i-1}^{2}} + \frac{1}{R_{i}^{2}} + \frac{1}{R_{i+1}^{2}}\right)$$
(19)

where  $R_{i-1}$ ,  $R_i$  and  $R_{i+1}$  are the radii of the circumcircles of the triangles  $P_{i-2}P_{i-1}P_i$ ,  $P_{i-1}P_iP_{i+1}$  and  $P_iP_{i+1}P_{i+2}$ , respectively,  $P_i$  is the regarded node, and  $P_{i-2}$ ... $P_{i+2}$  are its neighbourhood.

Applying the algorithm in this form to a stack of pictures leads to significant differences of the final contour from picture to picture. This is caused by the differences among the slides as well as stochastical noise, which remains in a minor form even after the noise suppression. To avoid this problem, the snake was modified to take into account the contours on adjacent slices. It is not a full-scale 3-D snake, but rather a 2.5-D one, where every contour lies on a single slice, but it has links to its neighbours. For this purpose, an additional deviation energy term  $E_{dev}$  was included. It bends every point of the snake to the nearest point of the same contour on the previous slide. This energy was calculated with:

$$E_{\rm dev} = \delta \cdot d^4 \tag{20}$$

where *d* is the distance between the given point and its correspondent on the previous slice in the cylindrical projection, and  $\delta$  is an algorithm parameter.

The starting contour on the first slide was given by a triangle inside the section of a scala. Its centre point was manually defined. The initial nodes of the snake were placed at a distance of three pixels from each other. For every further slide, the final contour of the previous slide was used as the starting contour. During each iteration step, the total energy minimum for every point including the deviation energy was achieved by finding the least-energy candidate along a normal to the curve. The maximum distance from the initial position of the given point was a parameter of the algorithm. After several steps, the process reached an energetically stable status and went to the next slide. Thus, the active contour goes through the scala to be segmented, fitting itself to its changing shape on every slice.

#### 2.2.4. Surface smoothing and reconstruction

In spite of adding the links between the snakes of adjacent slices, the resulting surfaces still showed distortions, which could appear due to the remaining noise and imperfections of the segmentation algorithm. To remove these artifacts, a simple floating mean algorithm was used. For every point of the surface, it collects the positions of all points in a cuboid-shaped surrounding of given dimensions. The mean of these positions is the new position of the point.

For adequate removal of one type of artifact, viz., the stripes on the scalae laying parallel to the  $r-\varphi$  plane in the cylindrical coordinate system, the smoothing was computed in the cylindrical coordinate system. The positions of the points taken into consideration were not absolute positions, but rather relative to the centre of the contour.

The borders of the segmented scalae were marked by a predefined value, isolated into separate stacks and transferred into "Amira" (Mercury Computer Systems, Inc.).

The study was performed in accordance with the German Prevention of Cruelty to Animals Act and permission was obtained from the Dresden Regional Council (24D-9168.11-1-2006-8).

# 3. Results

but not in a.

The improvement of the micro-CT resolution down to 4  $\mu$ m allowed visualizing the membranous structures of the cochlea. Fig. 4

shows the comparison of CT images of the cochlea received with different resolutions. Fig. 4b with a resolution of  $4 \mu m$  shows an essential improvement concerning especially the visualization quality of the Reissner's membrane compared to Fig. 4a with a 10  $\mu m$  resolution.

The results of the anisotropic diffusion algorithm are presented in Fig. 5. It shows that the noise has strongly decreased in comparison to the initial image, while the membranes are not significantly damaged. Of particular importance is that the intensity gradient of the noise has decreased. This prevents the subsequent active contour algorithm from stopping at noise particles.

Fig. 6 shows slices of a transformed stack corresponding to 90°, 180° and 270°. The shapes of the scalae sections are nearly constant, which allows the application of the snake homogenously to all stacks. The same object in the Cartesian coordinate system is shown in Fig. 7 for comparison.

A sample of the segmentation of the scalae with active contours is shown in Fig. 8. The membranes have been reconstructed even in those regions where they are hardly visible.

The reconstruction with "Amira" returned a geometry model presented in Fig. 9. This model can be exported into VRML format for use in a CAD software as a basis for further mechanical modeling.

# 4. Discussion

The results of the geometrical modeling of the cochlea provide not only a substrate for the understanding of macro- and micromechanical processes of hearing, but also present an important source for the development of therapeutic as well as preventive methods against a number of inner ear diseases. Other possible

250 μm Fig. 4. Two cochlea specimens under same magnification recorded with different resolutions: (a) 10 μm. (b) 4 μm. Reissner's membrane is clearly visible in b (arrows)





Fig. 5. Anisotropic diffusion results. (a) Image in cylindrical projection at 90°. (b) Image after smoothing by anisotropic diffusion filter.



Fig. 6. Cylindrical projection results. (a)  $90^{\circ}$  (b)  $180^{\circ}$  (c)  $270^{\circ}$ .

applications are the construction of new designs of electrodes for cochlear implants and the design of strategies for pharmacological treatment of the cochlea.

Currently, cochlear processes cannot be investigated completely with existing measuring techniques. Numerical simulations using mathematical procedures, such as finite element method or finite voulme method, remain the most promising approaches to understand physiological processes and their pathology. With these approaches, fluid-structure interactions can be simulated numerically as a multi-field problem.

The investigation of cochlear mechanics under normal and pathological conditions requires a reliable geometrical model of



Fig. 7. Changes in the topology of the scalae in the Cartesian coordinate system with increasing distance from the central axis of the cochlea. The fusion of scalae crosssections is indicated by arrows.



Fig. 8. Segmentation with active contours. (a) Image in cylindrical projection at 120° denoised with anisotropic diffusion. (b) Image after segmentation with active contours.

the inner ear, which considers all morphological changes of the cochlear structures.

There are very few noninvasive methods for the investigation of biological micro-structures, which are capable of simultaneously imaging bones and soft tissue. Modern computer tomography techniques allow the visualization of tiny objects with a resolution of down to 4  $\mu$ m and, thus, are comparable in their quality with conventional light microscopy. Therefore, this method can be referred to as "X-ray microscopy" (Vogel, 1999).

However, in case of biological objects as small as cochlear membranes consisting of few layers of cells, the application of this method is only possible with additional staining of the soft tissue. One solution to this problem is additional treatment of the cochlea with salts of heavy metals, e.g. OsO<sub>4</sub>. Osmium tetroxide is a standard fixing and contrasting substance in electron microscopy (Furness and Hackney, 1986; Mascorro and Bozzola, 2007). Its ability to penetrate deeply into soft tissues allows their visualization.



Fig. 9. 3-D visualizations of the scalae made with "Amira" using images segmented with active contours under "IPTools". (a) Cross-section through the model. (b) Scalae with one slice of the original image. (c) Surface model of the scalae returned by the surface generator.

The improved visualization of the membranes is an important requirement for the creation of geometric models of the cochlea. However, this achievement itself is not enough for this purpose due to speckle noise on the images and the complicated helical structure of the cochlea.

# 4.1. Denoising

The segmentation of raw micro-CT data is problematic due to speckle-like noise, which complicates the search for the intracochlear membranes. The noise does not allow identifying the membranes correctly with segmentation algorithms, which tend to recognize speckle-like noise as real structures due to its high gradients and relatively high intensity. Therefore, an image denoising algorithm is required, which preserves fine structures. For this purpose, the anisotropic diffusion appeared to be a good choice due to its careful handling of significant edges.

In spite of the advantages of the anisotropic diffusion method, a serious problem is the parameterization of this algorithm when working with a stack of images. Initially, the algorithm is controlled by three independent parameters: A,  $\lambda$ , and the number of iterations. As the computational time for a stack is not negligible, the choice of parameters by trial and error is time-consuming. On the contrary, fast processing is essential for practical purposes. Thus, an automation procedure is helpful for the automatic computing and/or for controlling these values.

# 4.2. Segmentation

A segmentation of every single scala is required for the threedimensional reconstruction of the intracochlear structures. The snake algorithm is a good choice for preservation and reconstruction of weak boundaries. The specific shape of the scalae makes the segmentation difficult when using the snake method in the Cartesian coordinate system. The sections of the scalae have different topology on slices that are located far from the cochlear axis shown in Fig. 1. Additionally, the sections of one scala fuse with each other at a certain distance from the axis (Fig. 7). To avoid this, the stack was transformed into a cylindrical coordinate system. In this case, the cutting plane always passes through the central axis of the cochlea. Thereby, fusion of the sections of the scalae does not occur, they vary very slowly when following them through the stack because they are almost normal to the curve of the respective scala (Fig. 6).

Considering the results of a 2-D active contour algorithm processed on a stack of images, differences may occur between the final contours on adjacent slices. This results in rippled surfaces of the scalae after reconstruction, although these are known to be smooth. The ripples seem to be caused by remaining noise on the slice as well as in the  $\varphi$  direction (stochastic changes of intensities of pixels with equal r and  $z_c$  coordinates between slices). To address this problem, two methods are proposed in this study: the first method is to link snakes on adjacent slices by inserting an introvert tension between them. Thus, this new procedure creates pseudo-3-D or 2.5-D snakes. It removes most crude errors, but also makes the algorithm much more stable when the snake follows a scala through the stack. With the 2.5-D snake, it becomes possible to segment a scala on an entire stack in one single process. With regard to the number of slices in the cylindrical projection (0.5° step delivers 720 slices), it influences working efficiency positively.

The second method – the surface smoothing – was applied after the first one. Although there exist implemented algorithms of surface smoothing with floating mean, the difference in the implementation used in this study was that it allows building a mean not only from a cube, but also from a cuboid with variable side lengths defined by the user. This is important for the scalae in the cylindrical coordinate system because the bordering surface is curved much more in the *r* and  $z_c$  directions than in the  $\varphi$  direction. Under these circumstances, it is more convenient to take a floating mean cuboid extended in the  $\varphi$  direction, as it would smooth folds laying across the scalae, while the small curvature details of scalae cross-sections would hardly be harmed.

#### 4.3. 3-D reconstruction

The segmentation of the cochlear scalae delivers information in the form of a set of snake knots. To ensure that the data are usable for building mechanical models based on the segmentation results, it is necessary to translate them to a common data format. Thus, a



Fig. 10. A structured surface mesh of the cochlea derived from the results of this study.

geometrical 3-D model of the scalae and bones needs to be constructed. The software required for this task must be capable of not only visualizing the models, but also of exporting the data in general formats. Therefore, the commercial software "Amira" was chosen for this study. With this program, it was possible to create geometric models from stacks of TIFF images and transform them to VRML files. This file format can be read by most CAD and meshing software. Here, the information about points on the surface was used to generate a smooth structured surface mesh in cylindrical coordinates (Fig. 10). The smoothing and interpolation of the data was accomplished with Matlab (MathWorks Inc., Matlab R2007b, 2007).

#### 5. Conclusion

Images from micro-CT can be used to visualize and 3-D reconstruct microscopic biological objects close to the cell level. This requires the impregnation of soft tissue of the specimen with salts of heavy metals. In this study, it allowed for the first time to visualize completely the fine membranous labyrinth of the inner ear, without mechanical distortions. For further modeling, a method of semi-automatic 3-D reconstruction was developed. It consists of:

- 1. denoising with the anisotropic diffusion;
- 2. reconstruction of the membranes with active contours;
- 3. creation of 3-D geometrical models with "Amira";
- 4. use data for a structured surface mesh.

The proposed approach is generally applicable. It also permits the processing of native images obtained with other methods (MRT, TEM, etc.), which are subjected to strong speckle-like noise and/or weak staining of delicate structures. The processing algorithms applied to the particular object of this research (cochlea) showed satisfactory results, fulfilling the requirements for a high-quality 3-D reconstruction. The geometrical models of the cochlea obtained here can be used for its further biomechanical modeling.

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## Appendix A

Factors of the tri-linear interpolation for the case of locally defined coordinates:

 $a = I_{111} - I_{011} - I_{101} - I_{110} + I_{100} + I_{010} + I_{001} - I_{000}$ (21)

$$b = I_{110} - I_{100} - I_{010} + I_{000} \tag{22}$$

$$c = I_{011} - I_{010} - I_{001} + I_{000} \tag{23}$$

$$d = I_{101} - I_{100} - I_{001} + I_{000} \tag{24}$$

$$e = I_{100} - I_{000} \tag{25}$$

$$f = I_{010} - I_{000} \tag{26}$$

$$g = I_{001} - I_{000} \tag{27}$$

$$h = I_{000}$$
 (28)

Here, the indices of *I* correspond to the coordinates in the local Cartesian x'-y'-z'-coordinate system (Fig. 3).

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