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Reconstruction accuracy of an automated serial cross-sectional preparation technique for morphological human temporal bone imaging

Abstract: Detailed knowledge about the three-dimensional morphology of the human cochlea and its intra-cochlear bony and soft-tissue structures is essential for development of new cochlear implant electrode carriers. A manual cross-sectional preparation and imaging technique, hereinafter referred to as "microgrinding", uses human temporal bone samples embedded in epoxy resin. This process was automated to shorten the time needed for preparation and to increase reproducibility. In this study, reconstruction accuracy of the automated microgrinding technique was determined.

Four assemblies of LEGO® bricks were used as artificial samples to analyze the resulting reconstruction accuracy of the whole procedure including embedding, preparation and image registration. The outer surfaces of the samples were measured using a portable coordinate measuring machine by manually choosing points on each surface. After embedding the samples in epoxy resin, following the protocol for human temporal bone samples, preparation using the automated microgrinding was performed with a slice thickness of 50µm. Pixel-spacing within an image was 11.5µm/px. The samples were identified within the dataset using threshold segmentation. Subsequently, points were manually chosen on each surface of the segmented samples. Planes, angles between planes and distances between corners were calculated for the physical and the digital samples. Deviation of the digitally derived measures compared to the physically derived ones describe the reconstruction accuracy of the automated microgrinding process.

Parallel planes had a deviation of $0.5° \pm 0.5°$ (range: 0 - 2.9°). Orthogonal planes, whose line of intersection is parallel to the direction of milled abrasion, had a deviation of $0.8° \pm 0.5°$ (range: 0 - 2.1°), whereas such orthogonal planes whose line of intersection is parallel to the documented sample surface had a deviation of $0.4° \pm 0.3°$ (range: 0 – 1.7 °). Distances between corners had an absolute deviation of 0.1 mm ± 0.1mm (range: 0 - 0.6mm), which corresponds to a relative deviation of 1% ± 1% (range: 0 - 6.5%). This study shows a highly accurate sample preparation using an automated microgrinding technique as an essential prerequisite for three-dimensional morphological imaging.

Keywords: microgrinding, histology, cochlea

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1 Motivation

Patients who suffer from a severe to profound hearing loss can be treated with a cochlear implant (CI) system, which is a neuroprosthesis. For the further development of the CI electrode carriers, which are inserted into the inner ear for stimulation, extensive knowledge about the three-dimensional (3D) geometry of the cochlea and its delicate intra-cochlear bony and soft-tissue structures is essential. In order to meet these requirements a manual serial cross-sectional preparation technique, hereinafter called microgrinding, was developed [1-3]. Following this method human temporal bone sample are cut into shape, dehydrated in ascending ethanol series and finally embedded in epoxy resin. The so derived cylinder-shaped samples are fixed within a custom-made sample holder for grinding of surface planes with a preset slice distance. Each surface is documented with a microscope before grinding to derive the next surface plane. Due to this high amount of manual interaction and the need for post-processing to increase image resolution [4] the process to derive the images is prone to handling-errors as well as cost and time consuming. The automation of the grinding process was established [5] to address these issues and increase reproducibility.

The aim of the here presented study was to identify the reconstruction accuracy that can be achieved with the automated microgrinding process.

2 Material and methods

2.1 Automated microgrinding setup

The automated microgrinding setup is based on a microtome (SM2500, Leica Biosystems Nussloch GmbH, Nussloch, Germany) which provides the smooth movement of the clamped sample from the milling to the documentation position. The microtome is combined with a milling cutter on a lifting head (SP2600, Leica Biosystems Nussloch GmbH), which allows for milling of the sample surface with defined slice thickness. A macroscope with a five-mega-pixel-camera (APO Z6 and DFC 420, both Leica Microsystems GmbH, Wetzlar, Germany) is mounted on top of the lifting head to document each new sample surface (see Figure 1).

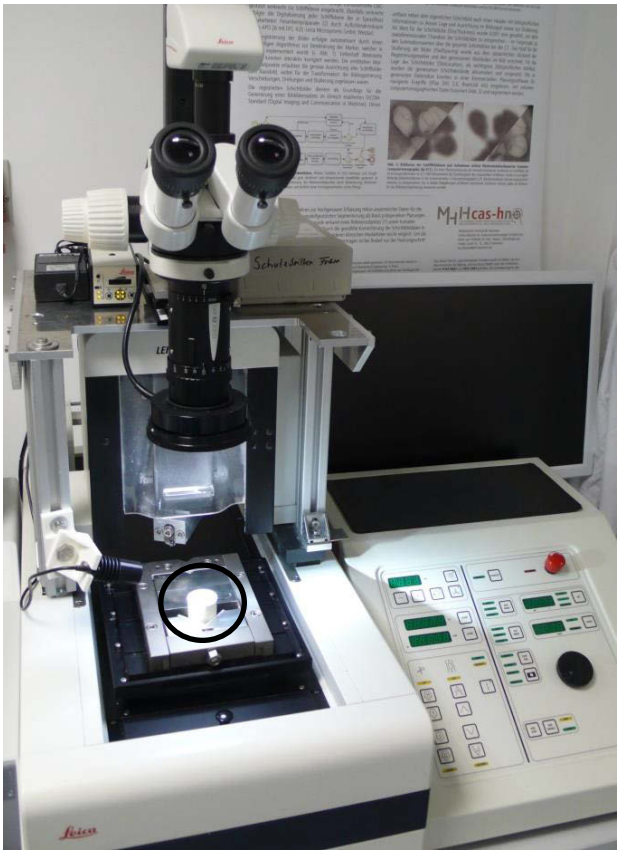

Figure 1: Automated microrinding setup. Microscope with camera is mounted on top of the lifting head of the milling cutter. A sample (black circle) is clamped within the microtome. The control unit is shown on the right hand side of the microtome.

2.2 Sample preparation

Four samples, each consisting of five LEGO® bricks (LEGO Group, Billund, Denmark), were used to analyze the reconstruction accuracy (see Figure 2). The outer surfaces of each LEGO® brick assembly were measured with a tactile coordinate measuring machine (FARO Gage, FARO Europe GmbH & Co. KG, Korntal-Münchingen, Germany) which has an accuracy of 0.018 mm. On the assembly of bricks 15 surface planes were defined and later used for analysis.

Figure 2: Artificial reference objects for determination of reconstruction accuracy of the automated microgrinding. Each sample was built from LEGO® bricks.

The measured samples were embedded using white epoxy resin and a cylindrical form. Finally samples were clamped into the automated microgrinding setup and processed with a slice thickness of 50 µm.

2.3 Reconstruction

The images were registered and exported as DICOM data set (Digital Imaging and Communications in Medicine), like previously described [2, 4]. These data sets were imported into a custom-made software (developed by G.J. Lexow) which enables for threshold-based segmentation and following measurement of individual point positions on the segmented surfaces of the digital Lego brick assembly (see Figure 3).

For each assembly we finally evaluated two sets of manually chosen points:

- the first derived by the tactile coordinate measuring machine on the samples before embedding,
- the second one derived by manually choosing points on the digitally reconstructed sample surfaces.

Following post-processing was done using Matlab. Each group of points derived from the same surface were used for calculation of a plane using normal and support vector.

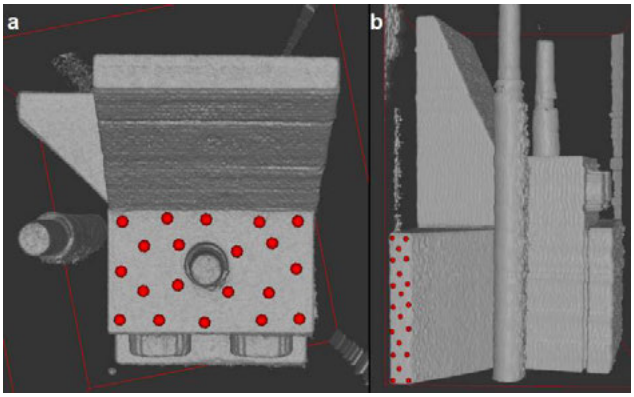

Figure 3: Top (a) and side (b) view on a processed, registered and segmented sample using the custom made tool. The pins are artificial registration markers. Red points show the manually chosen coordinates for two different surface planes (a,b) of the segmented sample.

Based on these planes, the angle between sample surfaces, the coordinates of each corner and the distances in between were calculated. The absolute error between the distances of corners derived from the physical sample compared to the measurements of the digital sample was used to analyze the reconstruction accuracy.

3 Results

Due to the magnification factor of the macroscope the pixel-spacing within a documented sample surface was 11.5µm/px. Parallel planes had a deviation of 0.5 ° ± 0.5 ° (range: 0 - 2.9 °). Orthogonal planes, whose line of intersection is parallel to the direction of milled abrasion, had a deviation of 0.8 ° ± 0.5 ° (range: 0 - 2.1°), whereas such orthogonal planes whose line of intersection is parallel to the documented sample surface had a deviation of 0.4° ± 0.3 ° (range: 0 – 1.7 °). Distances between corners had an absolute deviation of 0.1 mm ± 0.1mm (range: 0 - 0.6mm), which corresponds to a relative deviation of 1% ± 1% (range: 0 - 6.5%).

4 Discussion

The data set derived with the described process has an anisotropic resolution of 11.5 x 11.5 x 50 µm. The minimal possible slice thickness is 1µm, which theoretically enables isotropic resolution. However, reducing the slice thickness to get closer to an isotropic image resolution would increase the relative positioning error due to the positioning accuracy of the lifting head being 1 µm based on manufacturer information.

The measured reconstruction accuracy is in the same range than achieved manually [2]; although several differences in the accuracy of the measurement methods occur. The pixel spacing within a documented image was 13.7 µm for the manually processed samples [2], compared to 11.5 µm in the here presents study. The slice thickness was 100.4 µm, while the here presented process used 50 µm. Furthermore, the accuracy of the coordinate measurement systems to derive the geometrical data later used as gold standard for comparison with the segmented data varied substantially. The previous study by Rau et.al. 2011 [2] used a system with an approximated measuring accuracy of ± 8 µm, while the here presented study used another system with an accuracy of ± 18 µm.

Taken these variations into account, results are indicating that the automated procedure is useful as an appropriate alternative to the manual procedure in terms of spatial reconstruction accuracy and an improvement in terms of time and manual interaction.

Additional samples with embedded human and animal temporal bone specimen (processed during other studies, but with the here presented setup) provided insight that the presented embedding and processing protocol is applicable for samples with embedded bone instead of plastic material too. Further analysis has to be conducted to show whether dying of the whole sample before embedding may be possible and helpful to identify soft tissue structures within the cochlea when working with human temporal bone samples.

Author Statement

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Conflict of interest: Authors state no conflict of interest.

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