Non-Invasive High-Resolution µCT of the Inner Structure of Living Animals

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INTRODUCTION

In biomedical research, several animal models have been created to study the development and evolution of different diseases [1]. As is the case in the clinical situation, non-invasive imaging devices are required to analyse the expression of pathological influences and the effects of drug treatment [2]. Moreover, non-destructive imaging of living animals is a prerequisite for a reliable study of growth and development. In-vivo visualization has the unique feature that each animal will function as its own control during the evolution of a process.

Rats and mice are commonly used as small laboratory animals. Mice are extremely suitable because genetic manipulations have been successfully implemented in this species. Among currently used imaging devices for small, living laboratory animals are magnetic resonance imaging (MRI) and X-ray microtomography (µCT) [3]. MRI is often applied for the visualization of soft tissues whereas X-ray microtomography is most suitable for imaging bony structures. However, a major challenge in bioimaging of living animals is to achieve the highest possible spatial resolution.

In this article, we present the first in-vivo images obtained in living animals by a recently designed desktop scanner based on X-ray microtomography with a high spatial resolution [4, 5]. As a pilot study, growth and development of bones were analysed in rats and mice. First, the ankle of a rat leg was scanned and reconstructions were registered at different ages. In addition, the skull of the mouse was chosen for experimental bioimaging as it contains the brain as a vital organ which could be exposed to the X-rays in the in-vivo scanner, without introducing movement artifacts which could interfere with the high quality scans. 3D rendering was used to visualize growth in the rat leg and in the mouse skull.

MATERIALS AND METHODS

All measurements were made with a newly developed desktop scanner for high resolution X-ray microtomography (Skyscan 1076, Aartselaar, Belgium). Both the X-ray source (spot size 5 µm, energy range 20-100 keV) and the CCD camera (1k x 1k) rotated around the animal stage. A spatial resolution of approx. 15 micrometres was reached [4, 5].

Rats and mice (Wistar, CRL, WI and IOPS NMRI, Iffa Credo, Belgium) were anaesthetized with an intramuscular injection containing a mixture of Ketalar (Parke-Davis) and Rompun (2% Bayer). The animals were then fixed on a stage that could be moved into the scanner. The leg and the head of the animal were immobilized to avoid any movement. The scanner had three different fields of view: 40, 40 and 80 mm (2k x 2k, 4k x 4k and 8k x 8k) resulting in corresponding cross-sections (4 MB, 16 MB or 64 MB). During a single acquisition it was possible to scan preparations of one centimetre in length. Consecutive acquisitions made it possible to scan objects longer than 1 cm. The total length of the animal stage was 5 mm.

Figure 1:
Representative virtual cross-section in one plane through the ankle of a rat. Small trabeculae can be distinguished. A spatial resolution of approx. 15 µm was achieved. The large circle represents the reconstruction of the animal stage.

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200 mm. In scout view, the region of interest was chosen using the shadow X-ray image as a reference. Subsequently, data acquisition started. While scanning, visual control of the animal together with records of its breathing was obtained by a small built-in visual camera. All animals were scanned at the age of 5.5 and 7.5 weeks. The exposure times were 6 min for rats and 12 min for mice, as the latter were exposed twice to study a larger part of the skull.

After data acquisition, consecutive virtual slices through the region of interest were reconstructed. From these virtual slices, 3D models were created (ANT software, Skyscan, Belgium). Bony structures were matched at two different ages and three-dimensional models were superimposed to compare changes in growth.

RESULTS

A first major observation was that all animals survived repetitive scanning and stayed alive for several months after the experiment, without any visible health problems. This is particularly important as the brains of the mice were exposed twice. The radiation dose was reported to be 0.25-0.7 Gy per scan [6]. Six additional mice were scanned successfully.

After data acquisition, consecutive virtual cross-sections were reconstructed by applying a cone-beam Feldkamp algorithm. Initially, the ankle of a rat was scanned as shown in Fig 1. Figure 2 illustrates the result of scanning the head of a mouse. Bony structures in the skull and the teeth can be clearly distinguished by differences in grey values. The enamel is denser than the other parts. Without any additional staining, the detailed structure of soft tissues in the brain could not be seen.

These experiments prove that in-vivo scanning in mice is feasible without lethal consequences for the animals.

Figure 3: Two 3D reconstructions of part of ankle of the rat leg at two different ages. Green model: 5.5 weeks old (volume 7.3 mm³); yellow model: 7.5 weeks (volume 9.84 mm³).

DISCUSSION AND CONCLUSIONS

The reconstructions in the present study are the first images resulting from repetitive scanning by a new system for X-ray microtomography [4, 5]. The unique feature of this scanner is that a high spatial resolution (up to 10-15 µm) can be achieved. An increase in spatial resolution has always been a major goal in X-ray microtomography and in the visualization of living animals [3]. Yet, the radiation dose to which the animals were exposed in the present system was low enough to allow for frequent exposures [6].

An important conclusion from the present study is that small laboratory animals can be scanned on a regular basis without any visible health problems. This is in line with previous observations in invertebrate animals, where imaging by X-ray microtomography proved to be compatible with life and activity [7].

3D reconstructions are considered an important tool to understand spatial architecture and internal interactions within animals. In the present study, growth and development in bony structures were visualized over a period of time in rats and mice. Therefore, virtual cross-sections resulting in 3D rendering were different ages and growth was analysed. Besides an expected increase in calculated volume of the ankle, changes in shape were observed. Growth rate was 25% by volume in this particular bone. To further analyse the images, both models were registered. Figure 4 illustrates the procedure used to compare the 3D models in part of the rat leg. The orientation of the models was arbitrary to match as many details as possible, especially small trabeculae. Basically, matching was started on a visual basis. A given trabecula was recognized in both models and used as a landmark to superimpose the 3D reconstructions. The growth process proved to follow a non-uniform course.

As with the rat leg, the growth rate of the skull of a mouse was measured by matching 3D reconstructions, as can be seen in Fig 5. In a last series of experiments, the skull of a mouse was scanned twice with some small spatial overlap between the two scans to obtain 2 cm-long 3D models. The resolution of the basic grey-values in the virtual cross-sections proved to be high enough to allow for density thresholding. This is illustrated in Fig 6. The teeth were shown to have the highest density.
obtained without destroying the animals. This is in contrast to the painstaking efforts necessary to obtain 3D models when using classical, invasive techniques such as histology.

Matching of the 3D reconstructions allowed comparison of growth and development over a period of time, although a typical structure used as a landmark was still recognized on a visual basis. A challenge remains to develop further software to register different images more automatically. Yet, in the present study, changes in small structures such as individual trabeculae could be observed in this way, regardless of a significant extent of growth in the bone. This is of particular interest for further application in mice.

Regular scanning during the lifetime of small laboratory animals opens a unique possibility to control the expression of genes such as in bone mutations. Moreover, this bioimaging analysis is particularly relevant to study osteoporosis and related bone diseases in animal models.

Cross-sections with a number of significant grey values were obtained as was clearly demonstrated in the teeth of the mouse. The resolution of the basic grey values proved to be sufficiently high to allow density thresholding. This observation opens wide perspectives for a more quantitative analysis of mineral content in bony structures, especially in teeth.

In conclusion, the present observations make X-ray microtomography an attractive tool for diagnostic purposes in rats and mice. A range of new possibilities becomes available in biomedical research for imaging the evolution of development and disease together with possible treatment by drugs in small living animals with a spatial resolution sufficiently high to distinguish tiny structures.

REFERENCES