HIGH-CONTRAST 3D IMAGING
BIOLOGICAL TISSUES: EMBRYOS

Tomáš zikmund, Jozef Kaiser

CEITEC Central European Institute of Technology BRNO | CZECH REPUBLIC
Institute of physical engineering
Faculty Of mechanical Engineering
VUT Brno
Czech Republic

Marie Šejnohová

BRNO UNIVERSITY OF TECHNOLOGY FACULTY OF ELECTRICAL ENGINEERING AND COMMUNICATION
Biomedical engineering and bioinformatics
Faculty of electrical engineering
VUT Brno
Czech Republic

Josef Jaroš

Department of Histology and Embryology
Faculty of Medicine
Masaryk University, Brno
Czech Republic

Markéta Kaucká, Igor Adameyko

Karolinska Institutet
Department of Physiology and Pharmacology
Europan Molecular Biology Organization
Karolinska Institutet, Stockholm
Sweden

Abstract

Understanding developmental processes requires accurate visualisation and parameterization of three dimensional embryos. There exists a few methods for non-destructive whole-volume imaging of animal tissues. X-ray microtomography (microCT) has the potential to produce quantitative 3D images of small biological samples. The microCT imaging has been limited by the low inherent contrast of non-mineralized tissues.

Although X-ray contrast enhancement agents are used routinely in clinical radiography, only a few technique have appeared for imaging soft tissues in preserved animal specimens.

We present high-contrast imaging of embryonic tissues at histological resolutions using a commercial high-resolution lab-based microCT system. This is demonstrated on 14 day mouse embryo stained by phosphotungstic acid which produce overall contrast and differential tissue contrast. Using the staining method the microCT imaging is established as a useful tool for comparative developmental studies, embryo phenotyping, and quantitative studies of morphology.

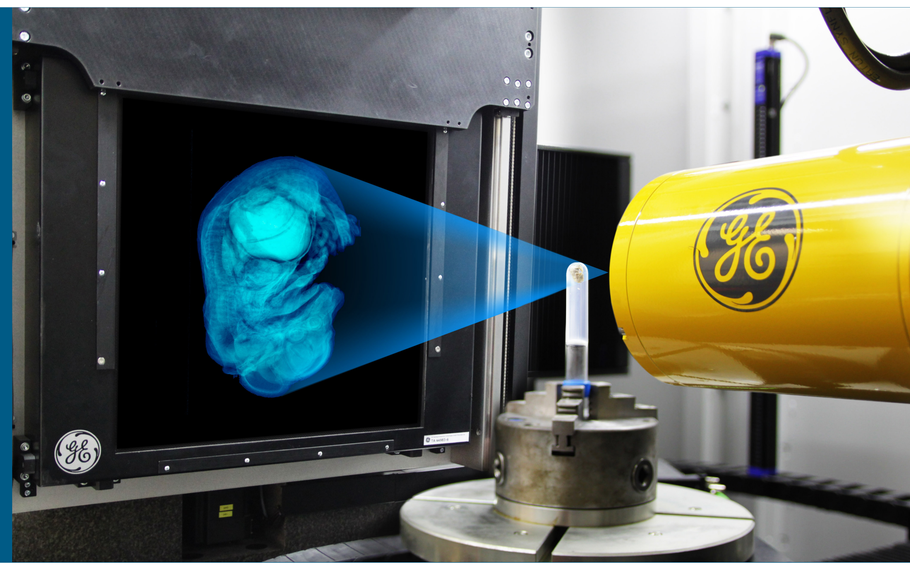
Methods

The 14th day mouse embryo was stained by phosphotungstic acid (PTA) [1]. This sample was embedded in the plastic tube using Agarose gel to provide mechanical stability.

The microCT analysis of the embryos was performed using system GE phoenix v|tome|x L 240, equipped with 180 kV/15 W maximum power nanofocus

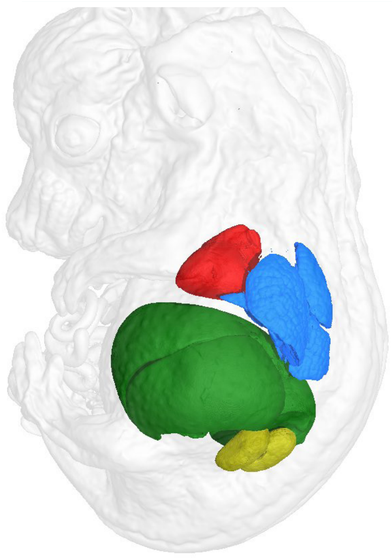
X-ray and high contrast digital array detector DXR250 with 2048 × 2048 pixel, 200 × 200 µm pixel size. The microCT scan was carried out in the air-conditioned cabinet (21◦C) at 65 kV acceleration voltage and 200 µA X-ray tube current. The exposure time was 900 ms. The voxel resolution of obtained volume was between 5 µm. The tomographic reconstruction was realized using GE phoenix datos|x 2.0 software.

The 3D- and 2D-cross section visualizations were performed with software VG Studio and Drishti. The olfactory system was segmented manually using Avizo.

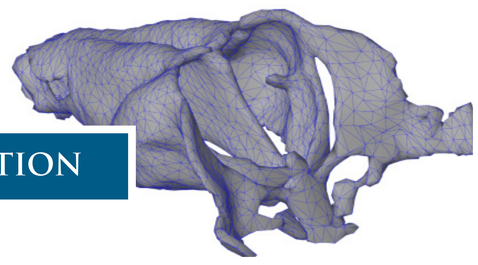


Projection of aligned CT data

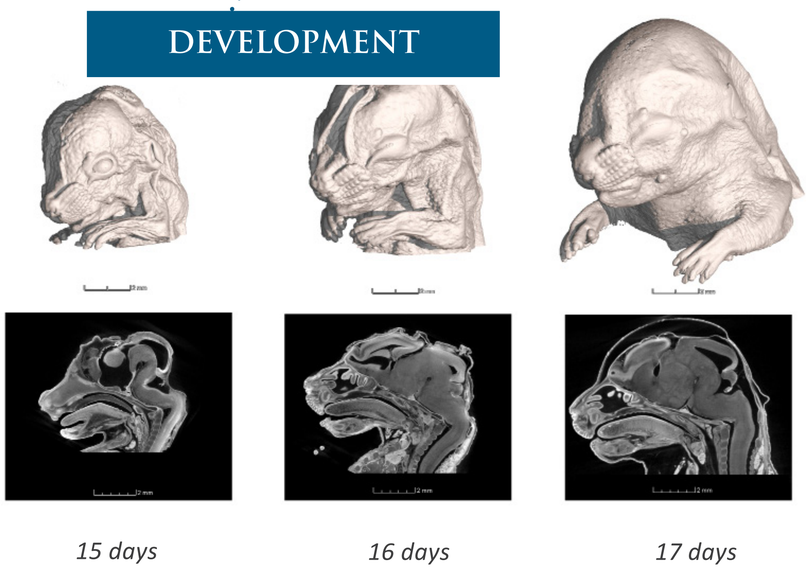
Orthogonal tomographic cross-sections



Various organs



Model of olfactory system



15 days *16 days* *17 days*

SUMMARY

The X-ray microtomography along with an appropriate soft tissue staining was established as a powerful imaging technique for ex vivo mouse embryo. The high resolution (5 µm) allows very detailed visualization of the inner structure.

We used PTA staining which produces overall contrast and differential tissue contrast for X-ray imaging. However this contrast agent doesn't affect the cartilage tissue, which makes the following segmentation of this part more difficult. For the segmentation of those parts we used the Avizo which has an advanced tools for the manual segmentation. The staining procedure produce also a certain amount of shrinkage which has to be taken in an account in the quantitative studies of morphology.

Acknowlegment

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Bibliography

[1] B.D.Metscher,MicroCTforDevelopmentalBiology:AVersatileToolforHigh-Contrast3DImagingatHistologicalResolutions, Developmental Dynamics 238 (2009) 632-640.