Industrial Tomography System for Answering Biological Issues: Development of the Mouse Embryo Face

Tomáš Zikmund, Dominika Kalasová, Markéta Tesařová, Jozef Kaiser

Brno University of Technology
CEITEC BUT – Central European Institute of Technology
Czech Republic

Lucia Mancini

Elettra - Sincrotrone Trieste S.C.p.A.2
Basovizza, Trieste
Italy

Josef Jaroš

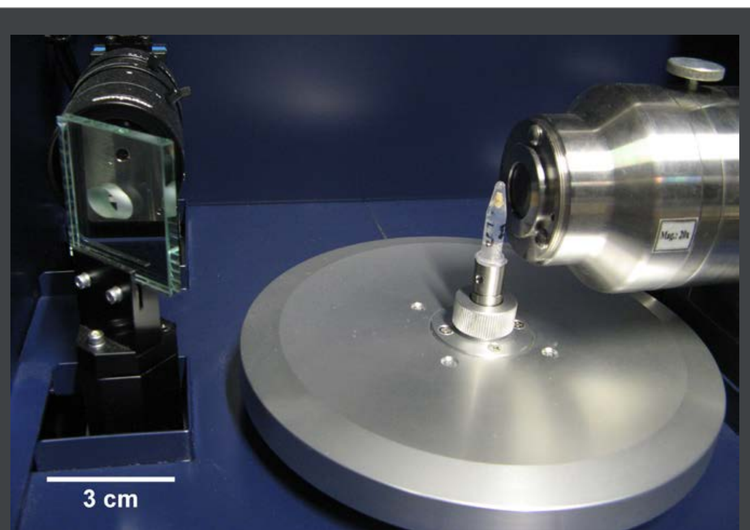
Department of Histology and Embryology
Faculty of Medicine
Masaryk University, Brno
Czech Republic

Markéta Kaucká, Igor Adameyko

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

David Paloušek, David Škaroupka

Faculty of Mechanical Engineering
Brno University of Technology
Czech Republic



Abstract

Understanding of some biological processes like a face development requires among others the high-resolution 3D imaging of increasingly complex cartilage in vertebrate embryos. During the face development, cartilages are produced in a variety of shapes and sizes making a convenient model system. X-ray computed microtomography (microCT) imaging is 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 techniques have appeared for imaging of soft tissues in preserved animal specimens.

Here we present a feasibility study on three different tomographic systems for high resolution and high contrast imaging of embryonic tissues. The industrial system equipped with flat-panel detector (GE phoenix v|tome|x L240) is compared with microCT system using synchrotron radiation (SYRMEP beamline of Elettra, Italy) and laboratory-based micro/nano CT system (Rigaku, nano3DX) based on Mo-target and CCD camera. The mouse embryos were stained by phosphotungstic acid which produced overall contrast and differential tissue contrast. Furthermore, we demonstrate the utilisation of the standard industrial tools (wall thickness analysis and 3D printing), which can help to understand the differences or similarities among different mouse embryonic development stages.

Introduction

Most of the bones in the body are formed by the well-defined ossification mechanism of cartilage structures at the end of the embryonic development. The research on cartilage development of the face is still an open topic [1]. One of the reasons is a substantial complexity of the structures in the head, including highly dynamic processes of the cartilage and bone formation, shaping and rearrangement. Bones and a few cartilage structures that exist untill adulthood fulfill important functions through the whole life and also they determine the shape of face and head.

Development of craniofacial parts is often studied using mouse embryos as a model system. Differences in mutation phenotypes affecting the cartilage structures of nasal capsule are investigated in embryos from 13 days to 18 days of embryonic development. Between these developmental stages the size of mouse embryos varies from 5 mm to 20 mm in average (Fig. 1). During the investigation of face development, high-resolution imaging techniques are required for observing tiny details in transformation of a cartilage.

Figure1: Mouse embryos at days 13 (left) and 17 (right) .

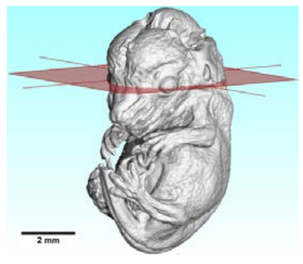


Figure 2: E14.5 visualized in VG Studio. The red plane represents the plane of cross-section of embryos in the Fig. 3, 4.

X-ray computed microtomography (microCT) has the potential to produce high resolution 3D imaging in a non-destructive way. However, X-ray based tomographic imaging of soft tissues is constrained by the low intrinsic X-ray absorption and lack of established contrast agents. Variety of contrast agents is used for contrast enhancement of such tissues [2].

The choice of the optimal microCT device strongly depends on the field of application. Many factors contribute to the final option, such as sample size and composition, required spatial and contrast resolution, scanning volume and time, available time and resources, etc. [3].

In this paper, we present results on tests of synchrotron radiation-based microCT (SRmicroCT), industrial microCT and laboratory nanoCT systems for the imaging of the embryonic soft tissue. We discuss the advantages of all used CT systems and demonstrate that the industrial CT system is an appropriate device for the development study of the cartilage tissue in a mouse embryo head. For this kind of study, we also show the usefulness of the common industrial techniques like a wall thickness analysis and 3D printing.

CT devices

GE phoenix v|tome|x L240

- 180 kV/15 W maximum power nanofocus X-ray tube
- flat panel detector DXR250 2048 px × 2048 px

RIGAKU nano3DX

- X-ray CCD camera 3300 px × 2500 px
- optical head with 2.5x magnification
- Mo rotatory target

SYRMEP beamline, Elettra

- monochromatic or white beam configuration
- energy in range 8 keV–38 keV
- CCD camera

Experimental details

Embryos at 12.5, 13.5, 14.5 stages of embryonic development (denoted E12.5, E13.5, E14.5) were stained with phosphotungstic acid (PTA) and embedded in agarose gel. The staining of the samples was based on modified protocol by Brian Metscher [2] and described in details in [4].

	acceleration voltage [kV]	exposure time [ms]	time of scanning [h]	linear voxel size [µm]
GE phoenix v|tome|x L240	60–65	750–1000	2.0	4.0–6.5
RIGAKU nano3DX	50	700	1.8	4.3
SYRMEP beamline, Elettra	9	1200–1800	0.6–1.8	9.0

Results

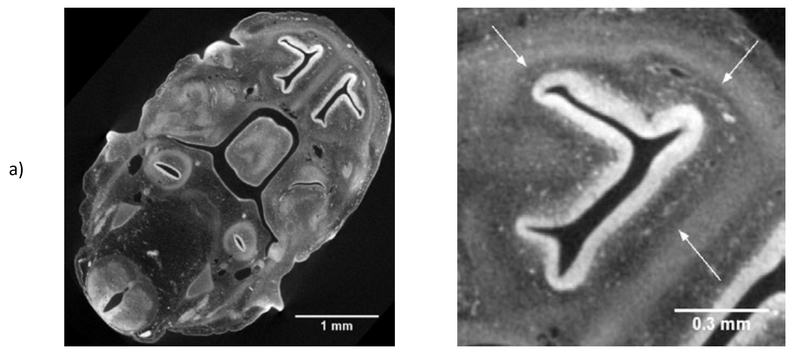
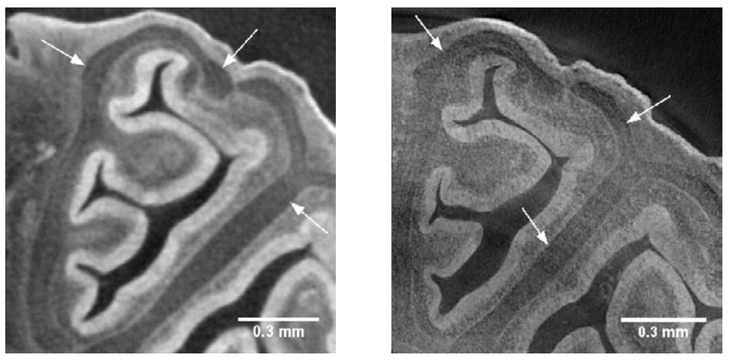


Figure 3: Comparison of microCT scans of heads of mice embryos from different systems – transversal views in the left column, details in the right column. The mesenchymal condenstation is pointed by arrow. a) E13.5, GE phoenix v|tome|x L240, b) E12.5, RIGAKU nano3DX, c) E13.5, SYRMEP beamline, Elettra synchrotron, monochromatic configuration.



(a) (b)

Figure 4: Details of cartilages in transversal views of E14,5. The olfactory system cartilage is pointed by arrow. (a) SYRMEP beamline, Elettra synchrotron, white beam configuration, (b) GE phoenix v|tome|x L240.

Discussion

The obtained result of the industrial system (Fig. 3a, Fig. 4b) showed the high quality data. In CT data there are no visible artifacts and cartilage is sufficiently distinguishable. Despite the cartilage is not properly stained the surroundings of soft tissue is making it visible enough for segmentation. This technique is characteristic by sufficiently intense X-ray spectrum from tungsten anode and large field of view. Therefore, the scanning time was reasonable. The resolution which is around 1/1000 of the real diameter of the sample, is good enough for a subsequent image processing.

The CT data from nanoCT station (Fig. 3b) are produced with low contrast and with scatter and beam hardening artifacts. This is because of tungsten concentration inside of the soft tissue is too high for the Mo X-ray spectrum. A small field of view (7 mm × 9 mm) was applicable only for the smallest embryo. To reach a voxel resolution under 1 µm the sample would have to be smaller than 1 mm in diameter.

Figure 5: Embryo after scanning in white beam.

Finally, one sample was scanned by SRmicroCT in monochromatic beam configuration (Fig. 3c) that allows phase contrast imaging. This would be very helpful for differentiation of the cartilage or mesenchymal condensation. Nevertheless, at the SYRMEP beamline the achievable spatial resolution with this configuration was comparable to the industrial system. That is why we decided to carry out a further phase-contrast SRmicroCT measurement with white beam configuration (Fig. 4a), which showed more detail compared to the industrial system (Fig. 4b). The embryos got blue (Fig. 5), probably because of high-intensity radiation, but appearently this didn't affect the sample morphology at the level of spatial resolution used in this experiment. Hovewer, this phenomenon will require deeper investigation.

Analysis and visualization

The cartilage tissue belonging into the olfactory system was segmented. The volumetric data of a segmented mask were transformed to a polygonal mesh (STL file) which describes the outer boundary of the region. The polygonal mesh, which consists of triangles, is a digital geometrical representation of the real object. The wall thickness analysis within VGStudio software was performed on polygonal model to show differences or similarities among thicknesses of the cartilage tissue in different embryo development stages. The results are shown on the polygonal mesh by a colour coded map in Fig. 6. Segmented olfactory cartilage in STL format was printed on ZPrinter 650 (Peak Solutions, USA) in 50:1 scale (Fig. 7).

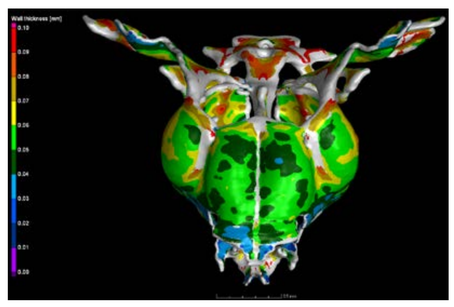


Figure 6: Wall thickness analysis applied to the segmented nasal capsule of mouse embryo. Colours show different thickness on 3D model.

Figure 7: The printed upscaled (50:1) model of olfactory system cartilage from the face of the embryo.

Conclusion

Three different microCT systems were tested for imaging the inner structure of mice embryos. The evaluation was focused on the cartilage of the olfactory system. RIGAKU nano3DX device showed availability only for small samples. Their tungsten-based staning is too strong to get good signal. Furthermore, the size of the mouse head doesn't allow us to reach significantly better resolution against other systems.

The data from industrial system GE in Laboratory of computed tomography CEITEC BUT and data from the SYRMEP beamline of Elettra showed up to be complementary. With respect to comparable results with synchrotron data and accessibility of the device, GE phoenix v|tome|x L240 is the most convenient system for visualization of cartilages of mouse embryos. On the other hand, when the younger stages include the mesechynmal condestation the SRmicroCT is appropriate due to the higher contrast and spatial resolution thanks to the possibility to use phase contrast imaging.

Subsequently, the nasal capsules of embryo's heads were segmented. The wall thickness analysis in VG studio software was applied to study the evolution of thickness of cartilage. The 3D models were exported to STL format and used to create plastic models on 3D printer for direct visualization.

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