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

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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 soft tissues in preserved animal specimens.

Here we present a feasibility study of three different tomographic systems for high resolution and high contrast imaging of embryonic tissues. The industrial system equipped with flat-panel 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 of different mouse embryonic development stages.

Keywords: industrial tomography, lab-based nanotomography, synchrotron-based microtomography, mouse embryo, wall thickness analysis, 3D printing

1 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 remain till adulthood fulfill important functions through the whole life together with determining the shape of face and head. Development of craniofacial parts is often studied using mouse embryos as a model system [2, 3]. Differences in mutation phenotypes affecting the cartilage structures of nasal capsule are investigated in embryos at 13-18 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.
Optical, later electron microscopy has been used for imaging 2D histological sections for a long time. To meet the requirements for more general high-resolution visualization of entire sample 3D imaging techniques have been developed. Optical microscopy enables collection of optical sections through thicker samples via concocal or episcopic microscopy, even with time-lapse [4]. In confocal microscopy, a pinhole is placed at the confocal plane of the objective to eliminate out-of-focus light [5]. Episcopic microscopy is based on autofluorescence of each tissue slice. However, these methods are costly and time-consuming [6]. Optical projection tomography is able to produce high-resolution 3D image of specimens transparent to visible light, but only of the size of several millimeters [7]. Magnetic resonance imaging is a well-established method for imaging of morphology of soft tissues [8]. The image is created by applying magnetic field and radio waves on the sample. This method is restricted by achievable resolution, which is about 25 µm [6]. X-ray computed microtomography (microCT) [9] has the potential to produce high-resolution 3D imaging in a non-destructive way also for opaque objects. 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 [10].

The choice of the optimal microCT device strongly depends on the field of application. High-resolution microCT is employed in various industrial applications as well as in many fields of science. 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. [11]. Conventional laboratory high-resolution industrial microCT systems require robust, stable devices which are able to hold big and heavy samples. The large field of view is achieved by employing large area detectors such as flat panels (400 × 400 mm²). The nature of samples often requires high-voltage X-ray sources tubes, which produce X-rays in a cone beam geometry, beam with high penetration power. The cone beam allows high geometric magnification and/or enables large scanning volume. The use of such a machine is relatively easy, quick and cost-effective [11].

However, thanks to the technical improvement of tomographic components (X-ray tubes and detectors), lab-based CT systems are able to reach even better resolution, which is nowadays hundreds nanometers. These devices are usually called nanoCT systems. They lose some advantages of bigger industrial systems like a large field of view and high energy X-ray spectrum, but they found their place for biological specimens or light materials (polymers, composites and powders).

Synchrotron radiation-based microCT (SRmicroCT) [12] produces a high-intensity coherent nearly parallel X-ray beam with energies from 5 keV up to energies of the order of 100 keV. High-flux beam provides excellent contrast resolution. The vertical field of view is smaller than in industrial devices, and the spatial resolution is detectors-limited. The high resolution optics coupled to CCD or CMOS cameras is typically employed. Either white-beam or monochromatic beam can be used for SRmicroCT experiments. In case of monochromatic beam, the precise value of the energy can be chosen and therefore no beam hardening artifacts appear [13]. A high spatial coherence of X-ray beam allows utilization of phase-contrast imaging techniques [14].

Post-processing and analysis of acquired data isn’t less important. There is a variety of available softwares, which provide further analysis of data. VG Studio MAX is a software focused on the industrial needs. It allows the quantitative analysis of internal microstructure, such as pore or inclusion analysis, wall thickness analysis, coordinate measurements, fibre orientation analysis, etc. These analyses are of much use in industry, but they also find their application in science [15].
In this paper, we tested synchrotron radiation-based microCT, 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 development study, we also show the usefulness of the common industrial techniques like a wall thickness analysis and 3D printing.

2 Experimental details

2.1 Sample preparation

Embryos at 12.5, 13.5, 14.5 (E12.5, E13.5, E14.5) stages of embryonic development were stained and embedded in agarose gel. Staining of the samples was based on modified protocol by Brian Metscher [16] and described in details elsewhere [17].

2.2 Measurements

The cartilage structures in the head of mice embryos in different evolution steps were studied. Mice embryos were scanned using three different microCT devices. The resulting images were compared in order to determine the best one for further measurements and analysis.

The experiment using synchrotron radiation was performed at SYRMEP (SYnchrotron Radiation for MEdical Physics) beamline at the Elettra synchrotron radiation facility in Trieste, Italy. The horizontal acceptance covered by the front-end light-port is 7 mrad, that implies that beamline provides, at a distance of about 23 m from the source, a laminar section X-ray beam with an useful area of about $160 \times 5 \text{ mm}^2$. In monochromatic configuration, a double Si(111) crystal system in Bragg configuration is employed allowing to tune the X-ray energy in the range 8 keV -38 keV. In white beam mode, the scans were carried out with an exposure time/projection of 1.2 s - 1.8 s and an angular step of 0.1° over a total scan angle of 180° or 360° depending on the sample size. The voxel size of the reconstructed volume was in the range 2.0 µm - 2.8 µm. In monochromatic mode, energy of X-rays was 9 keV and voxel size was 9 µm. The reconstruction of the tomographic data was performed using a custom developed software SYRMEP Tomo Project written in IDL® language.

The two other measurements were performed in X-ray micro and nano computed tomography laboratory in CEITEC BUT. All the samples were scanned on a GE phoenix v|tome|x L240 device, equipped a 180 kV/15 W maximum power nanofocus X-ray tube and high contrast flat panel detector DXR250 with $2048 \times 2048 \text{ pixel}^2$ and $200 \times 200 \text{ µm}^2$ pixel size. The microCT scans were carried out at 60 kV - 65 kV acceleration voltage and 170 µA - 230 µA X-ray tube current, the exposure time was 750 ms – 1000 ms. The time of scanning of one sample was around 2 hours. The voxel size of obtained volume was in the range of 4 µm – 6.5 µm depending on size of an embryo head. The tomographic reconstruction was realized using GE phoenix datos|x 2.0 3D computed tomography software.

The CT measurement of early stage embryo was performed on RIGAKU nano3DX device (Fig. 2). This machine is equipped with $3300 \times 2500 \text{ pixel}^2$ X-ray CCD camera and Mo rotatory target working at acerelation voltage of 50 kV and current 24 mA. The optical head with 2.5x magnification was chosen to reach the field of view at $7 \times 9 \text{ mm}^2$. Due to the low signal a bining 2 was set which determined the linear voxel size of the resulting CT data at 4.32 µm. The time of scanning was 1.8 hours with exposure time of 7 s.
2.3 Analysis of data

The cartilage tissue belonging into the olfactory system was segmented by drawing the mask in every slice of the stack. A smoothing procedure was used to get rid of the steps from the “slice by slice” segmentation. 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 was performed on polygonal model to show differences or similarities among thicknesses of the cartilage tissue in different embryo development stages. This analysis within the VGStudio searches the opposite surface by sending a measurement line orthogonal to the current surface. The surface area of the opposite surface is taken into account for the end point of the measurement line is defined by a search cone. The search cone was set at 30°. The results are shown on the polygonal mesh by a colour coded map.

2.4 3D print

Segmented olfactory cartilage in STL format was printed on ZPrinter 650 (Peak Solutions, USA) in 1:50 scale. The detailed procedure of manufacturing of 3D models is described in [17].

3 Results and discussion

As the study of the face development is focused on the olfactory system cartilage, we chose the tomographic cross-section including this part (see Fig. 3) for the quality data evaluation. The histograms of the all presented images were equalised to make the best contrast for the cartilage tissue against the other soft tissue. The different embryonic stages were tested on each system because of their different possibilities. The cartilage is not completely developed in younger stages of the embryo and instead of it there is a mesenchymal condensation. This tissue has a better staining ability but it is less distinguishable from the other tissue.
Figure 3: E14.5 visualized in VG Studio. The red plane represents the plane of cross-section of embryos in the Fig. 4.
The measurement result of the industrial system (Fig. 4a, Fig. 5b) 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. This technique is represented by sufficient tungsten X-ray spectrum and large field of view. Therefore, the scanning time was reasonable. The resolution which is around 1/1000 of the real diameter, is good enough for a subsequent image processing.

The CT data from nanoCT station (Fig. 4b) 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 × 9 mm²) 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.

Finally, one sample was scanned by SRmicroCT in monochromatic beam configuration (Fig. 4c) which 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. 5a), which showed more detail against the industrial system (Fig. 5b). The embryos got blue (Fig. 6), probably because of high-intensity radiation, but apparently this didn’t affect the sample morphology at the level of spatial resolution used in this experiment. However, this phenomenon will require deeper investigation.

Figure 6: Embryo after scanning in white beam.

When the CT data are already generated and cartilage system is segmented and transformed to the STL format it can be used for other study. The wall thickness analysis (Fig. 7) can easily help to understand what mechanism is behind the growing of the face. In addition, the segmented cartilage system can be printed in different scales or colors (Fig. 8). This can be helpful for understanding of the model 3D morphology and educative purposes.

Figure 7: Wall thickness analysis applied to the segmented nasal capsule of mouse embryo. Colors show different thickness on 3D model.
4 Conclusion

Three different microCT systems were tested for imaging the inner structure of mice embryos. The evolution was focused on the cartilage of the olfactory system. RIGAKU nano3DX device showed availability only for small samples. Their tungsten-based staining is too strong to get good signal. Furthermore, the size of the mouse head doesn’t allow to reach significant 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 condensation the SRmicroCT is convenient due to the higher contrast and spatial resolution thanks to the possibility to use phase contrast imaging. Consequently, 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 a plastic models on 3D printer for direct visualization.

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