The use of
Micro and nano computed tomography
for imaging soft tissues in embryos

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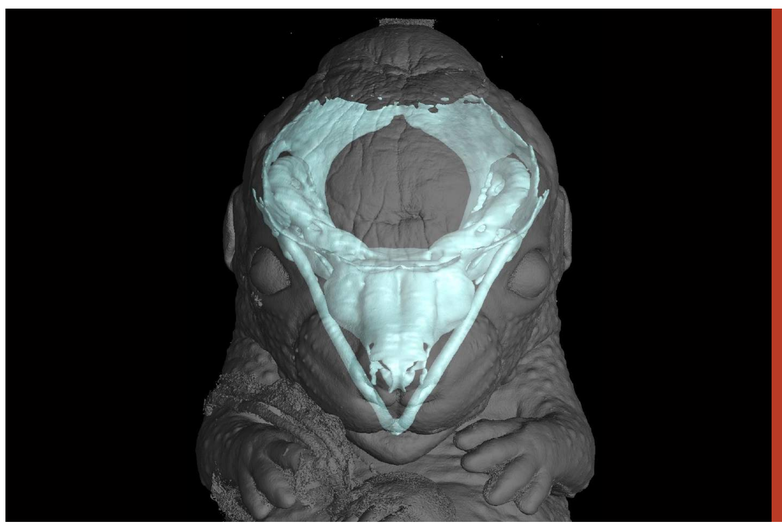

Introduction

Imaging of soft tissues is crucial for understanding chemical and biological processes inside the living organisms. This is especially important to study initial formation during embryonic development [1-3]. Such imaging requires the best possible resolution and high demands on preparation of the samples. However, 2D image does not contain all sufficient information about observed structures. Recently, imaging techniques were improved considerably to allow extend traditional imaging techniques to 3D imaging.

One of the convenient tool for 3D imaging of embryonic samples is X-ray Computed Tomography (CT). It is a non-destructive method with spatial resolution up to 1µm. However, CT imaging has been limited by low contrast of soft biological tissue. X-ray source shows very similar absorption for unmineralized structures. In this case phase-contrast tomography or staining agents are applied.

In this work the high contrast 3D data of soft tissues in embryonic samples were obtained by staining with iodine and phosphotungstic acid and subsequent tomographic analysis. This brings new possibilities for exploring and understanding the processes inside the evolving body.

Here we present the application of X-ray micro and nano Computed Tomography as a powerful tool for 3D imaging in biology.

I X-ray Computed Tomography

X-ray Computed Tomography (CT) is a non-destructive method for imaging of inner structure of materials. X-ray micro and nano computed tomography with high spatial-resolution has the same principle as CT machines used for medical scanning. The sample is placed between the X-ray tube and the detector. A lot of projections from different angles of rotation of the sample are recorded. From these projections, slices through the sample are reconstructed to get 3D data. The obtained data is a map of X-ray intensity and phase change.

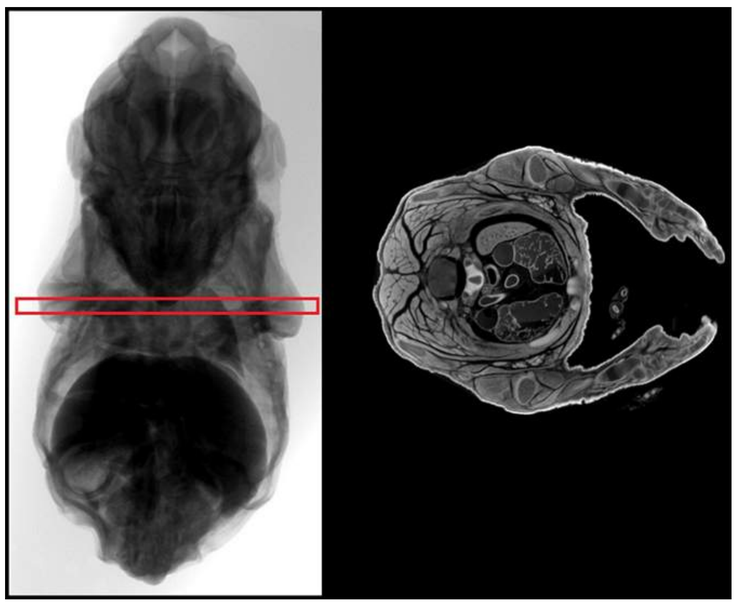

Fig.1. Tomographic reconstruction - Left image shows 2D X-ray projection and red area represents one pixel row. Right image is a corresponding reconstructed slice of mouse embryo.

II Staining of soft tissues

CT imaging method has been limited by low contrast of soft un-mineralized tissues. From that reason, contrasting agents are used for staining of sof tissues of biological samples. ***Phosphotungstic acid*** (PTA) was utilized as standard histological technique for light and electron microscopy due to its capability to increase the contrast of different type of tissues. PTA confers strong contrast for samples as embryos, when attached to the collagens, fibrils and to other proteins. Other agents used for contrasting for CT are compounds with elements with higher atomical number as ***iodine*** or ***osmium***.

Finding an appropriate staining protocol with good penetration to whole the sample (figure 2) and with no shrinking of the tissue (figure 3) is crucial for successful tomographic measurement.

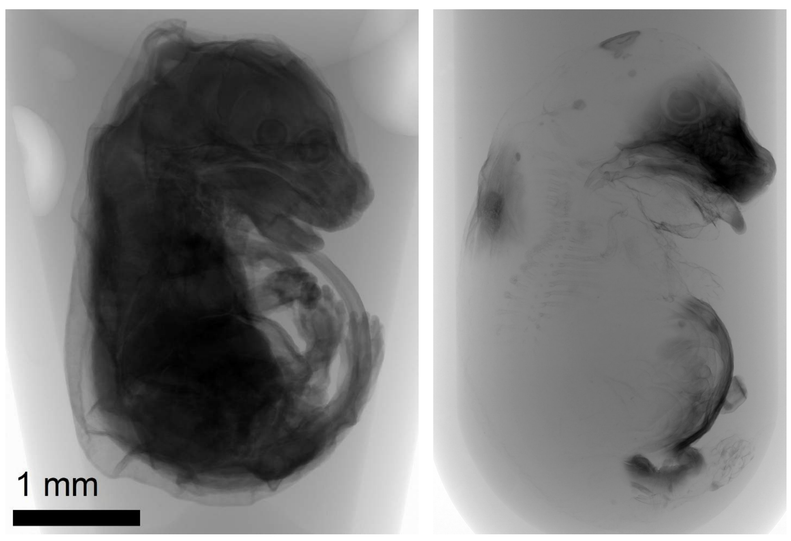

Fig.2. Staining - X-ray projection of the stained embryo (Left) in comparison with embryo that was not stained properly (right).

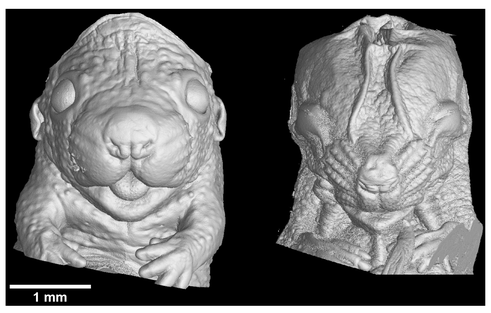

Fig.3. Shrinking - Embryo that was shrinked (right) during the staining procedure in comparison with non-shrinked embryo (left).

III 2D ---> 3D

Why is it needed to image in 3D and 2D data are not enough? Figure 4 shows example of 3D visualization of craniofacial part at two different mouse embryos (mutant and its control). It is not clear on 2D tomographic slices if there are differences between mutant and conrol. 2D section is always dependent on the plane of the slice. However, 3D segmentation of cartilage clearly shows what are the main differences between the samples.

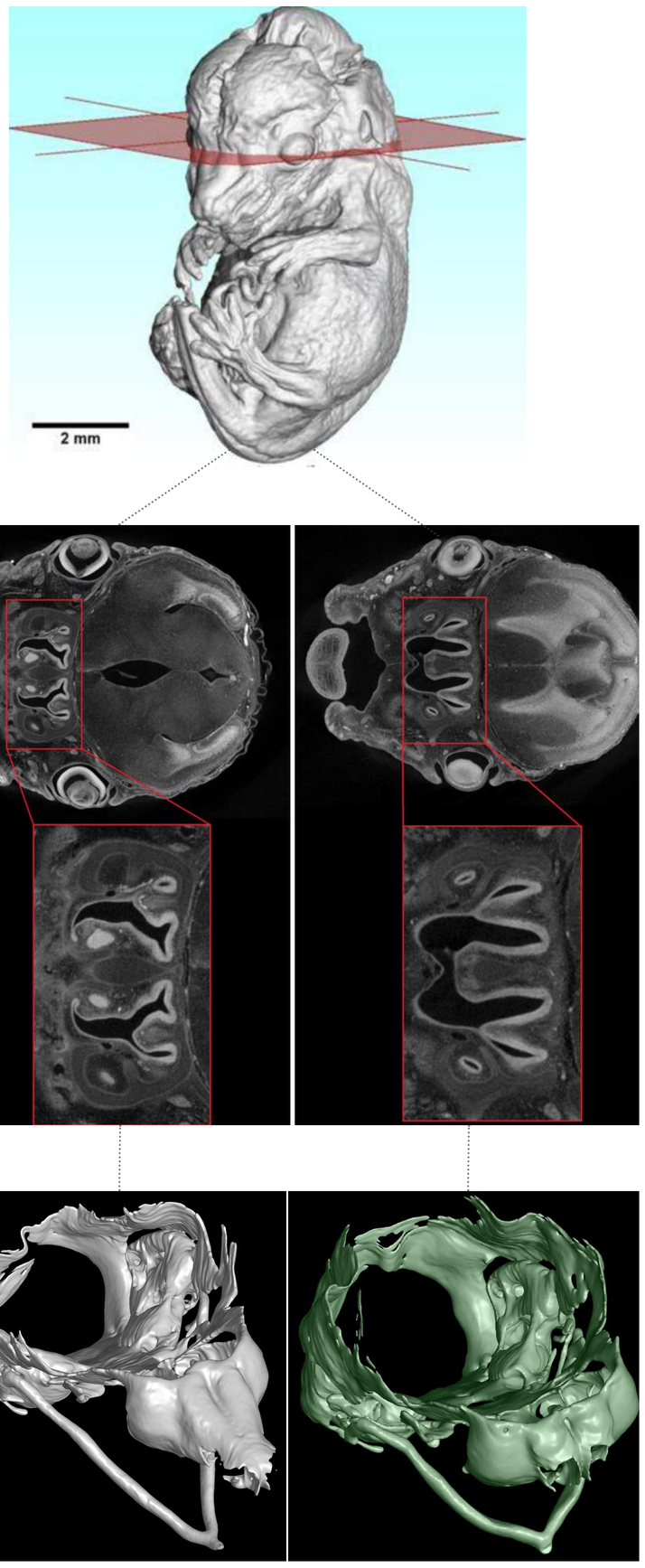

Fig.4. Benefits of 3D imaging - The top image shows plane of interest and corresponding 2D tomographic slices at two different samples with area of interest at craniofacial cartilage. The bottom images show 3D segmentation of whole cranium.

IV Analysis of 3D models

After tomographic measurement and reconstruction, another important part is data segmentation, analysis and interpretation of the 3D data. To show finest changes between segmented 3D models, various mathematical method can be applied. Figure 5 shows shape comparison using aligment of models by least squares method and subsequent finding of normals from each point of triangular 3D mesh.

Another example of analysis is finding the wall thickness of the craniums at mouse embryos (Figure 6).

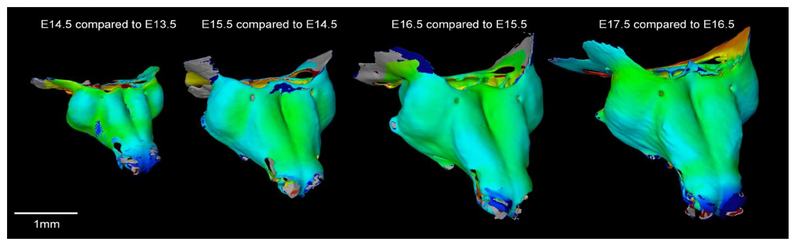

Fig.5. Shape comparisons - Analysis of change of the shape of nasal capsules of mouse embryos [3].

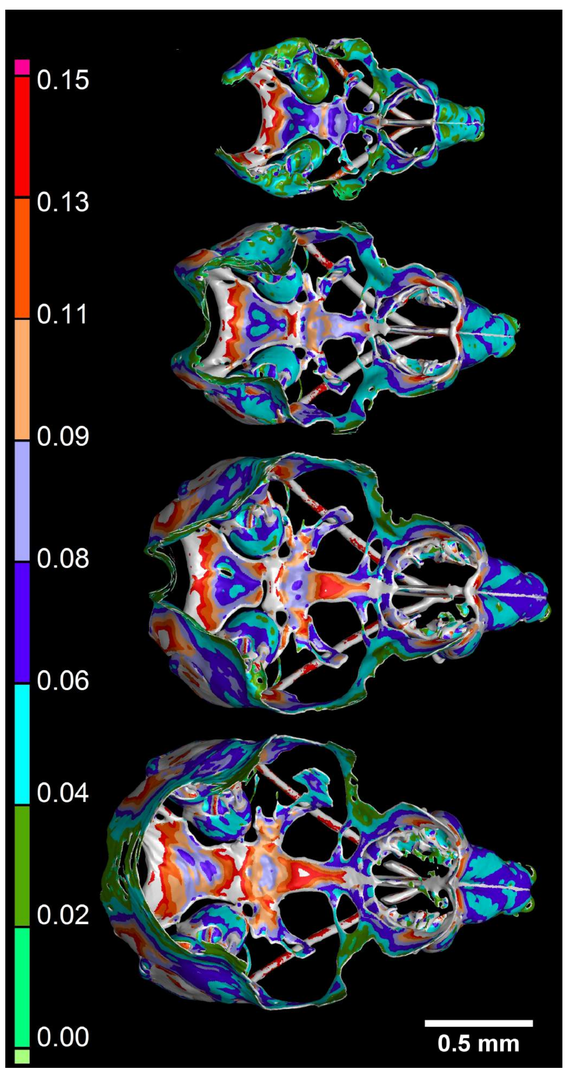

Fig.6. Wall thickness analysis - Thickness of the cartilage of craniums at mouse embryos. Developmental stage E14.5 - E17.5 [3].

V Multi-purpose approach

Big advantage of CT method is large variety of tissues that can be detected and visualize. Moreover, all structures are visible in one 3D model, so the samples can be studied in whole context of the body with studying internal structure in parallel.

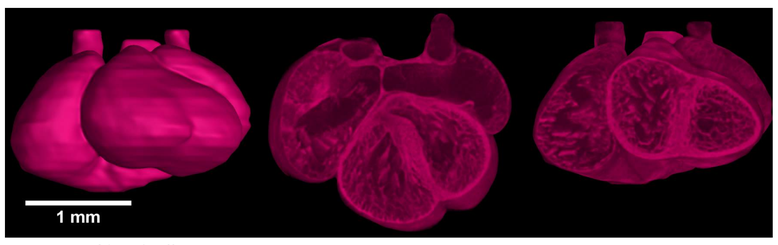

Fig.7. Inner structure of heart - shape of the heart of mouse embryo with virtual clipping planes showing 3D structure.

References:

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