3D ANALYSIS OF MOUSE EMBRYO BY X-RAY COMPUTED TOMOGRAPHY

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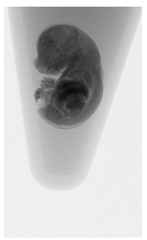
ABSTRACT

X-ray Computed Tomography (CT) provides 3D imaging various types of samples. The state of the art of CT systems originally designed for industrial applications can contribute to our understanding on how the shape diversity observed among living organisms is defined and controlled during development and growth. CT has proven capability to explore embryonic formation in different developmental stages with high-resolution and in a non-destructive manner. The complexity of biological structures often requires a comprehensive approach to compare shapes, sizes and volumes of studied structures. Due to comparison of various mutant mouse models, data processing and quantification of morphological 3D changes can be challenging.

In this work, we introduce industrial microCT system as a new suitable tool for comparative developmental studies, embryo phenotyping, and morphology studies. This is demonstrated on selected structures and organs of mouse embryos by means of shape comparison, volume determination, thickness measurements, position/orientation analysis and 3D printing.

WORKFLOW OF MOUSE EMBRYO microCT ANALYSIS

Embryo staining
- phosphotungstic acid (PTA)
- iodine solution



Embryo after staining
(X-ray projection)

MicroCT measurement

GE Phoenix v|tome|x L 240
(GE Sensing & Inspection Technologies GmbH, Germany)

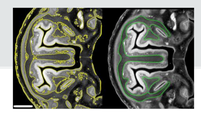
Tomographic reconstruction

Tomographic slice of mouse embryo

Data processing

VOLUME GRAPHICS
Avizo
MATLAB
GOM Inspect Professional

Segmentation
- manual
- automatic (segmentation algorithms, i.e. thresholding)



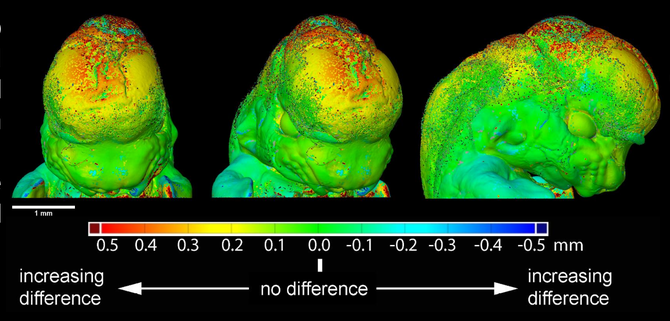
Comparison between automatic (yellow) and manual (green) segmentation. Scale bar 0.5 mm.

3D model creation

Data analysis:
Evaluation of various sample parameters (shape, size, volume)
Wall thickness analysis
Fiber orientation analysis

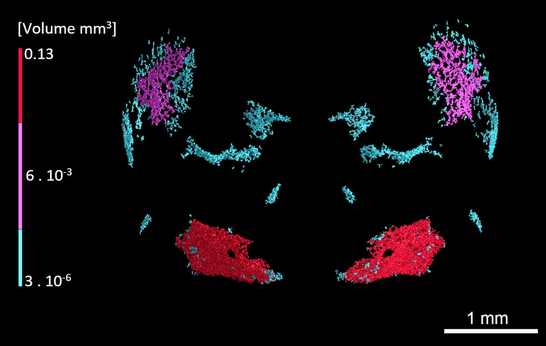
SHAPE COMPARISON

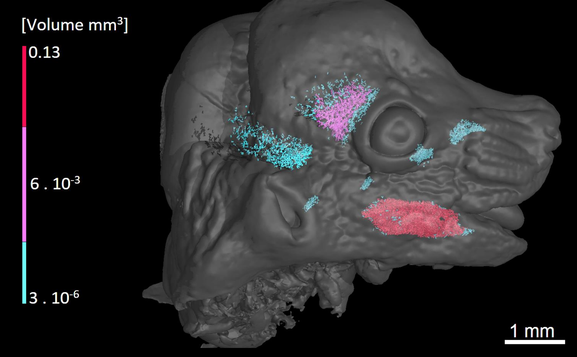
GOM Inspect software was used to compare the shape of the head between mutants and control embryos. The image show comparison at E12.5 developmental stage.
Green color significants no difference between the mutant and the control embryos.



VOLUME DETERMINATION – PORE / INCLUSION ANALYSIS

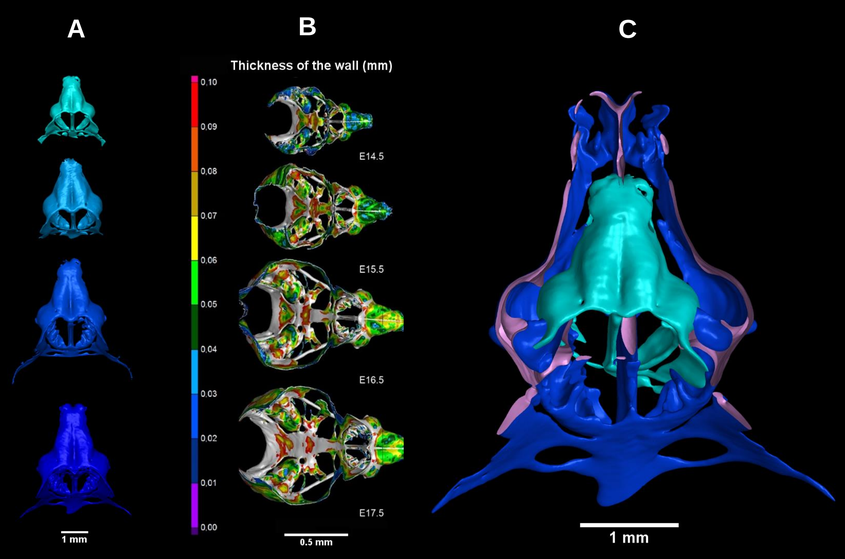
Head of 15.5 days old mouse embryo was scanned without staining in order to detect ossification centers in skull. Pore/inclusion analysis in VG Studio max 3.1 was used to evaluate interconnection and volume of ossified tissue. Colour scale defines volume of each connected system of ossified tissue.





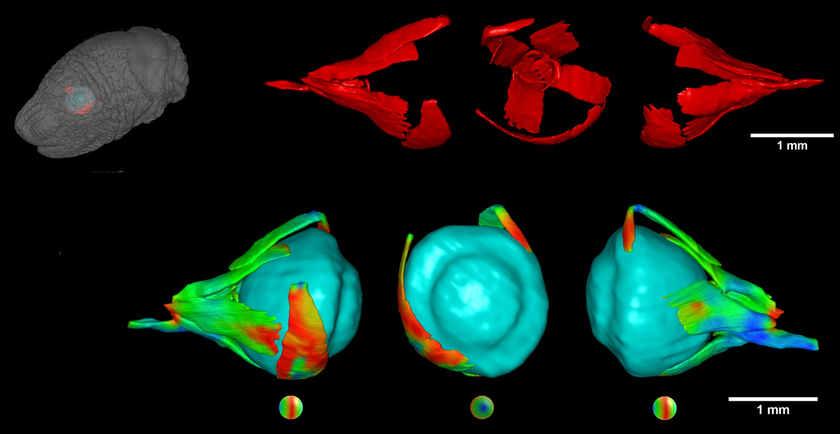
WALL THICKNESS ANALYSIS

3D models and wall thickness analysis of chondrocraniums at different developmental stages. A) 3D models of nasal capsules created from segmentation of the raw microCT scans of mouse embryos at developmental stages E14.5 to E17.5. B) Analysis of the cartilaginous wall thickness of the whole chondrocraniums at E14.5-E17.5 developmental stages. C) Comparison of E14.5 nasal capsule inside of E17.5 nasal capsule.



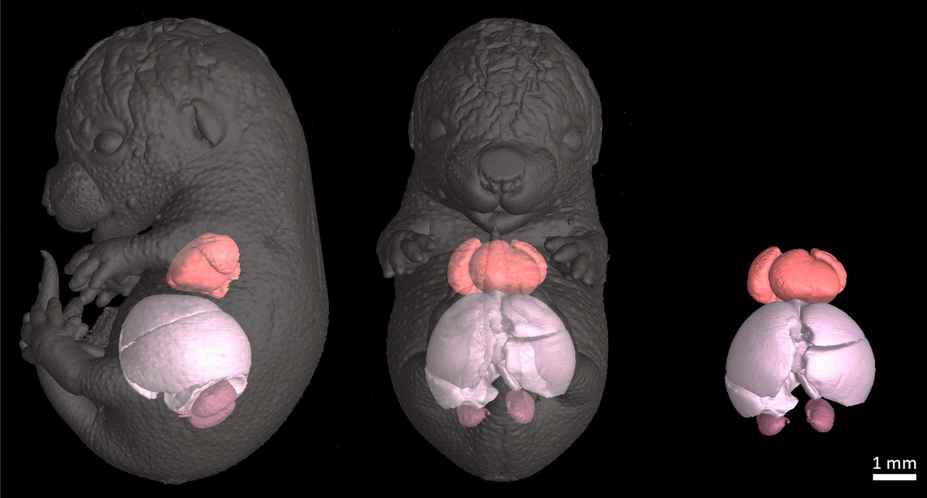
FIBER ORIENTATION – EXTRAOCULAR MUSCLES

3D Visualization of extraocular muscles and eyeball with its lens at mouse embryo E18.5. To understand shape-making process and molecular mechanisms at cellular level, it is important to know exact angles and orientation of forming fiber muscles.



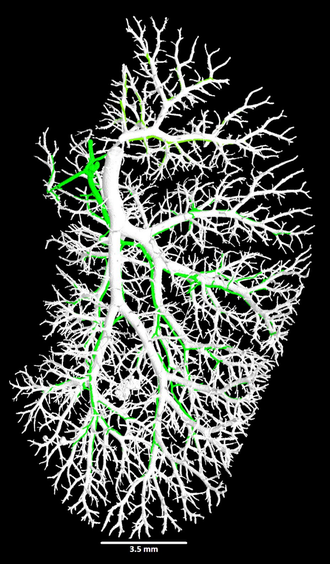
VISUALIZATION OF DIFFERENT PARTS AND/OR ORGANS

Intact 15.5 days old mouse embryo was scanned in microCT device. Quality of resulting data enables segmentation of organs and inner structures, segmented regions can be further analyzed eg. wall thickness analysis and pore analysis. As an example liver, heart and kidneys were segmented.



VISUALIZATION OF DIFFERENT INTERNAL SYSTEMS

MicroCT was applied for visualization of vascular and biliary system after injection of two synthetic resins (MICROFIL®). Visualization in 3D enables to study architecture of systems and the data processing allows comprehensive evaluation of system parameters: i.e. volume, length or the number of bifurcations and trifurcations.



3D PRINTING

Although several software-based methods for visualization of 3D data sets are available, having a solid model of the object under study provides additional opportunities to examine and understand the shape-organizing processes in the developing body. Here we show the full procedure of creating a real 3D object of mouse embryo nasal capsule, which includes staining and microCT scanning combined with advanced data processing and 3D printing.

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