Three-Dimensional Reconstruction of the Hindbrain Choroid Plexus Using X-ray Micro-Computed Tomography

Parobková V.[1], Kompaníková P.[2], Zikmund T.[1], Bryja V.[2], Buchtová M.[3], Lázňovský J.[1], Kaiser J.[1]

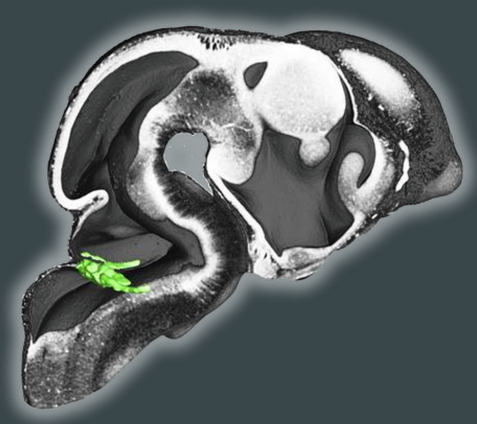
[1]Central European Institute of Technology, Brno University of Technology, Czech Republic

[2]Faculty of Science, Masaryk University, Brno, Czech Republic

[3]Laboratory of Molecular Morphogenesis, Institute of Animal Physiology and Genetics, v.v.i., Czech Academy of Sciences, Brno, Czech Republic

Introduction

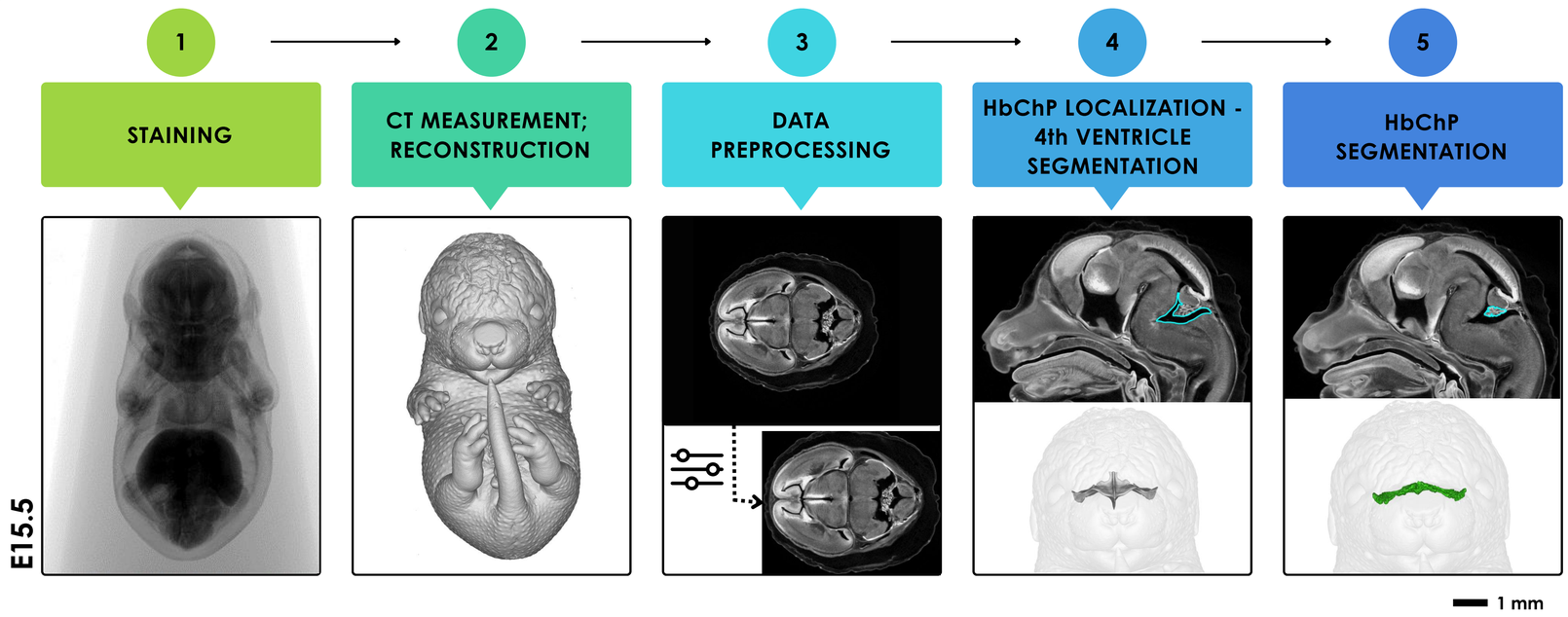
The Hindbrain Choroid Plexus (HbChP) is a pivotal player in the central nervous system, governing cerebrospinal fluid production, CNS homeostasis, and embryonic brain development. In our study, we introduce ChoP-CT, an innovative X-ray micro-computed tomography (micro-CT) methodology, to enable precise three-dimensional reconstruction of murine embryonic HbChP. This methodology involves sequential steps including staining, CT measurement, and advanced segmentation tools to delve into the intricate structure of the HbChP. Despite challenges arising from nuanced contrast between lateral branches and surrounding tissue, we incorporate manual segmentation for enhanced accuracy. The resultant semiautomatic algorithm generates a comprehensive 3D model of HbChP. ChoP-CT, a pioneering tool, facilitates detailed morphological analysis and empowers the scientific community by offering a 3D visualization of HbChP. By unlocking the structural dimensions of HbChP, this methodology opens avenues for in-depth investigations into its roles in brain development and health.



Materials and Methods

As the model organism, the generation of the **Cdk13$^{tm1a/tm1a}$** and **Cdk13$^{tm1d/tm1d}$** mouse embryos were selected along with the **Tmem107$^{-/-}$** mice line and their wildtype and knockout species in stages **E13.5, E15.5** and **E17.5** were further analyzed. Before the measurement, mouse embryos were stained in 1% I^2 in 90% MeOH solution to enhance the contrast of the soft tissues in µCT data. µCT measurement was performed on device GE Phoenix v|tome|x L 240 laboratory system equipped with 180 kV /15 W nanofocus tube, and the data were reconstructed using the professional software provided by the same provider. The HbChP was segmented semi-automatically using a self-written program implemented in MATLAB. The manual segmentation was held in Avizo software. The 3D morphology of HbChP was visualized in the VG Studio Max software, where the final model was completed and exported for further processing. Lastly, the Oculus Quest from Meta was used to access another experience from a 3D model in VR with the help of Simlab software selected for the model handling.

Segmentation

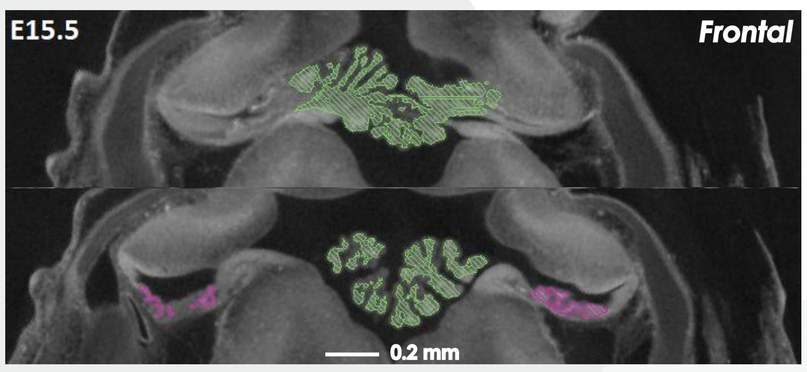


4th Ventricle segmentation Based on the reference model of the 4th ventricle downloaded from the publicly available atlas [1], the region was retrieved by registration of this reference to our binarized data containing the brain ventricular system. By this approach, the algorithm was able to separate the 4th ventricle apart from the complex ventricular system.

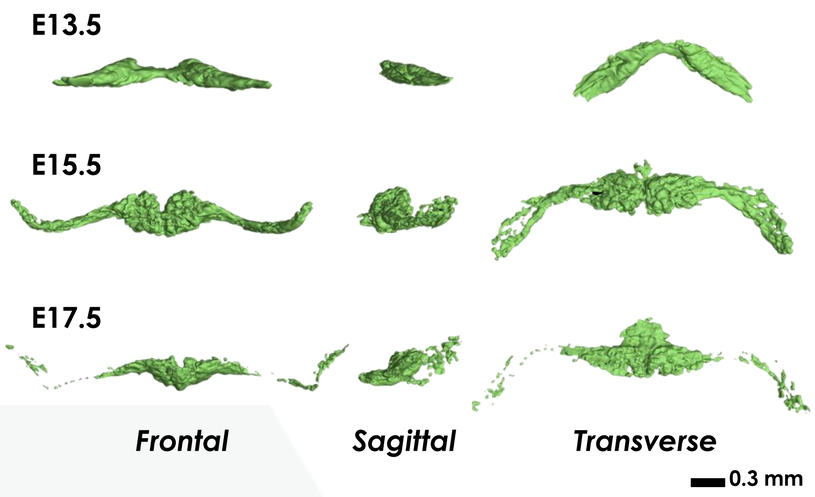
Choroid plexus To distinguish the HbChP within the 4th ventricle, several steps were taken. The HbChP stands out due to its high-intensity (bright) voxels and higher local range in image, which result from its complex structure. Therefore, the high-intensity voxels with a high range value were extracted from the ventricular region. Next, the biggest connected region formed from extracted voxels was selected, generating the primary mask of the HbChP. Lastly, the active contour model was applied on a primary mask to include all HbChP voxels and refine the segmentation. For the older embryos (>E13.5), the described algorithm couldn't include the lateral branches and therefore, their model were finished by manual segmentation.

Results

Semi-automatic approach The segmentation algorithm operates automatically during the early developmental stages of embryos when the HbChP is centrally located within the 4th ventricle and hasn't extended into the lateral part, specifically the foramen of Luschka. However, for more mature species, manual segmentation becomes necessary to include the lateral branches.



3D reconstruction of the HbChP Models for each developmental stage were produced. By E15.5, the HbChP is already prolonging into the foramen of Luschka on both sides. The central region ('cauliflower-like structure') densifies and becomes more complex along the development.

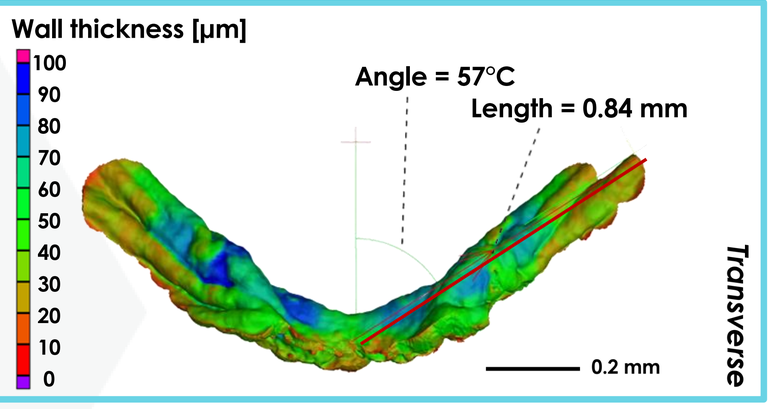


Virtual Reality Virtual reality was chosen to enhance the experience of the 3D models created from µCT data. The models were simplified and colored using VG Studio Max, and then uploaded to a VR viewer (Simlab).

AVAILABLE AT OUR VR POINT

Conclusion

A workflow was suggested for 3D rendering of HbChP using our publicly available segmentation tool called ChoP-CT. This approach offers valuable reconstructed 3D models of HbChP, aiding deeper exploration and comprehension of its intricate structure and its relation to potential organism malfunctions due to underdeveloped plexus. The model facilitates **quantitative morphological analysis**, including measurements like volume, growth angle, wall thickness, and lengths.



REFERENCES

[1] Wong, M. D., et *al*. (2012). A novel 3D mouse embryo atlas based on micro-CT. Development, 139, 3248–3256. 10.1242/dev.082016

ACKNOWLEDGEMENT

We acknowledge CzechNanoLab Research Infrastructure supported by MEYS CR (LM2023051) and the Grant Agency of the Czech Republic (21-05146S). V.P. is a holder of the Brno PhD talent scholarship, which is funded by the municipality of the city of Brno, Czech Republic.

Viktória Parobková

Laboratory of X-ray micro and nano computed tomography

Viktoria.Parobkova@ceitec.vutbr.cz

www.ctlab.cz

CEITEC – Central European Institute of Technology

Brno University of Technology

Purkyňova 123, 612 00 Brno, Czech Republic

www.ceitec.eu