# There is more than bone in your head: How to visualize both skull and brain with a single micro-CT acquisition

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## Aims

Down syndrome (DS) is a genetic disorder that affects the development of multiple organs, including the face, the bones and the brain, resulting in cognitive deficits. Recently, Epigallocatechin gallate (EGCG), a green tea flavonol, has shown potential to improve the cognitive and skeletal deficits in DS[1,2,3]. However, the possible integrated effects that the EGCG treatment may have in the morphology of the skull bones and the brain have not been properly evaluated yet. In this context, our aim is to develop a technique that allows the visualization of both the skull and the brain in a single acquisition, thereby reducing the costs and time of multiple acquisitions and obviating the need for offline co-registration. We optimized diceCT[4], a technique that uses iodine as a contrast agent to visualize soft tissues in a CT scan, to enable integrated and accurate mouse brain and bone shape and size analysis.

## Method

Whole-body micro-CT-scans with a reconstructed isotropic voxel size of 50 µm were acquired on a Skyscan 1278 microCT scanner. To enhance the visualization of the skull and the brain we tested three different staining approaches (perfusion, diffusion and a novel stereotactic injection approach) using Lugol ($I_3K$) as the contrast agent and different parameters and protocols. With the perfusion approach, we tested different injection rates, as well as different concentrations of Lugol and paraformaldehyde (PFA). Regarding the diffusion approach, we optimized the protocol considering the freshness of tissue, fixation time, incubation time in Lugol and frequency of changing to fresh Lugol. Finally, for the stereotactic injection in the brain ventricles, we optimized the volume and injection rate of Lugol, and the injection in either one or the two lateral ventricles, before and after fixation.

## Results

We could not reach homogeneous contrast enhancement in the brain via trans-cardiac perfusion in combination with blood-brain barrier disruption (Fig. 1A). For the diffusion approach, we optimized a protocol to obtain highest brain contrast with minimal shrinkage, allowing us to clearly analyze the brain volume and visualize different brain regions such as the cerebellum (Fig. 1B). We have been able to clearly visualize the brain ventricles (Fig. 1C), allowing whole brain and ventricle segmentation (Fig. 1D-F) using our novel stereotactic injection approach. The skull was always visible in all the approaches. Using a combination of both the stereotactic and the difussion techniques, a size and shape analysis of the brain, ventricles, mandible and skull becomes feasible.

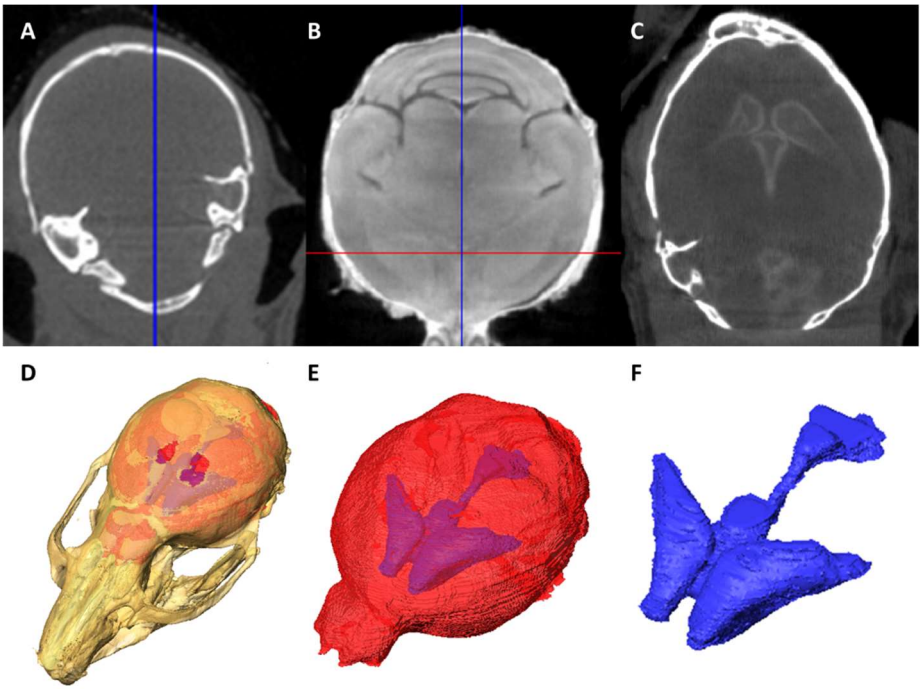


Figure 1: Different staining approaches for optimizing DiceCT scanning and visualize the skull and the brain in a single acquisition.Top row: A) Perfusion approach, B) Diffusion approach after 23 days in Lugol and C) Stereotactic injection approach. Bottom row: results from the stereotactic injection approach, showing different segmentations where the skull (yellow), the brain (red) and the ventricles (blue) are visible from one single scan (D), from which each structure can be visualized and analyzed separately (E,F).

## Conclusion

Although multimodal acquisition and co-registration of MRI and CT is currently the gold standard, we here propose two different novel approaches to visualize both the skull and soft tissue of the brain in a single scan. Although there is still room for optimization, our current approach is readily applicable for mouse model evaluation in Down syndrome as well as in broader fields such as Neuroscience and Developmental Biology.

## References:

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