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Automated whole-brain volumetric parcellation and cortical surface extraction of BigBrain2 using nnU-Net + FreeSurfer v8.1

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Introduction:

In 2013, we published BigBrain1, a high-resolution (20μm^3) histological 3D-reconstructed model of the human brain (Amunts et al., 2013). Over the past several years, focus has been directed towards advances in the reconstruction and analysis of BigBrain2 (Mohlberg et al., 2022; Lepage et al., 2023; Lewis et al., 2024; Mohlberg, Lepage et al., 2025).

However, it remains a notable challenge to use established automated brain-imaging pipelines - which have been validated and optimized for typical in-vivo MRI data (e.g., FreeSurfer, ~0.5mm^3-1mm^3) - to process brain volumes reconstructed from histological data at a substantially higher resolution (~100μm^3-200μm^3). Major difficulties include: sectioning artifacts, different tissue contrasts, staining imbalances, and sheer size of data, leading to sub-optimal performances, memory bottlenecks, and prohibitively long processing times.

In this work, we obtain a whole-brain volumetric parcellation of BigBrain2, together with the extraction of cortical surfaces, via an adaptation of FreeSurfer v8.1. Such a segmentation can provide regions of interest for higher-resolution analyses, such as FreeSurfer's hippocampal subfield or brainstem segmentations (Iglesias et al., 2015), or other external analyses.

Methods:

Successful cortical surface extraction is contingent upon accurate white / grey matter tissue classification, as well as proper subcortical masking.

Preprocessing:

nnU-Net classified volume: The nnU-Net algorithm (Isensee et al., 2021) was used to obtain a tissue classification for white matter, grey matter, layer-1, and background on the repaired histological sections every 100μm apart, defined from a training set of 77 sections 2mm apart. nnU-Net provides fast robust 2D segmentation insensitive to staining imbalances, unlike global 3D tissue classification. Where a 3D classical classification algorithm would fail, the deep-learning approach was suitably capable of distinguishing layer-1 of the cortex from white matter, despite both tissue classes showing identical cell-body stain intensities. A 3D classified volume at 100μm^3 isotropic resolution was then reconstructed from the aligned resampled 2D segmented images at 20μm^3, in order to serve as the tissue classification in FreeSurfer for the purpose of extracting the cortical surfaces.

Histological volume: To obtain the required standard initial FreeSurfer whole-brain subcortical segmentation (1mm^3), the original histological intensity volume was submitted to mri_synthseg, and the output inserted as needed into the FreeSurfer v8.1 -hires (150μm^3) recon-all pipeline.

Surface extraction:

Interventions to recon-all (including the inputs described above) allowed for the extraction of the cortical surfaces (~167k vertices per hemisphere), as well as produced a final subcortical segmentation, which was refined by the cortical surfaces at the resolution of the input volume.

Results:

Fig 1A shows nnU-Net classified volume (4 tissue classes, 100µm^3), with original histological volume for reference (100µm^3).

Fig 1B shows FreeSurfer white and gray surface extractions for BigBrain2.

Fig 1C shows automated FreeSurfer volumetric parcellation output (wmparc.mgz) for BigBrain2 (150µm^3).mri_synthseg - now default in FreeSurfer v8.1 - unavoidably internally downsamples all input to 1mm. Therefore, all sub-cortical delineations shown are at 1mm^3. Only the cortical white / grey segmentations are refined by the surfaces extracted at 150µm^3.

Fig 1D shows FreeSurfer hippocampal subfield segmentation output (150µm^3 within ROI defined at 1mm^3).

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Session Classification: Session 2: Mapping & Atlases