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3D reconstruction of BigBrain2: Expertise and new tools for the next generation of BigBrains

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BigBrain2 is a second BigBrain dataset that complements and builds on our expertise from the first BigBrain [1], providing new insights into variations between brains at whole-brain and cytoarchitectonic level. The brain (30-year-old male donor) was formalin-fixed, paraffin-embedded, and sectioned coronally (20 μm) into 7676 sections. Each section was stained for cell bodies (Merker stain). The sections were scanned at 10 μm in-plane (flatbed scanner) and 1 μm in-plane.

We have developed a new approach to assist the labour-intensive process of correcting the artefacts in the histological images, and, subsequently, to reconstruct the high-resolution 3D volume, with correction for staining imbalances. Despite a significantly improved wet-lab processing pipeline, sectioning and histological preparation of a whole brain at this thickness remains a challenging task, leading to a heterogeneity in the extent and severity of artefacts, rendering a fully automated repair process of all sections impracticable.

Initially, the 10 μm sections were resampled at 20 μm in-plane, to match the section thickness, and every fifth section (5-series) was repaired manually, ensuring data provenance tracking [2]. An initial 3D reconstruction at 100 μm has been created [3]. Remaining sections were processed sequentially; larger artefacts were identified and manually corrected [4]. For the remaining artefacts, each section was registered to the two nearest repaired sections of the 5-series, from which a virtual reference image was interpolated at the position of the target section. Smaller artefacts (e.g., missing data) were corrected by interpolating good tissue from the reference section in place of the missing tissue in manually identified areas.

To support the 3D reconstruction, tissue masks were created in an automated fashion using the nnU-Net algorithm [5], with a combination of global and local training sets. The approach was extended to obtain a tissue classification for white matter, grey matter, and layer-1 on the repaired histological sections, every 100 μm apart, using a training set of 77 sections 2 mm apart. Unlike global 3D tissue classification, nnU-Net provided a fast and robust 2D segmentation insensitive to staining imbalances and could suitably distinguish layer-1 from white matter, despite both tissue classes showing overlapping cell-body stain intensity distributions.

The masked repaired sections were aligned to the post-mortem MRI of the fixed brain in an iterative process by 3D registration of the stacked images to the MRI, followed by 2D registration of the individual images to the sliced MRI, while gradually increasing the complexity of 2D and 3D registration from rigid-body to affine to non-linear. These global iterations helped resolve the lower-frequency alignment errors causing 'jaggies', as were present in BigBrain1, and accounted for tissue compression and shrinkage during histological processing. Finally, section-to-section non-linear 2D alignment (without MRI) was performed to resolve high-frequency alignment errors. Optical-balancing was applied to correct for staining imbalances across the brain volume.

The new pipeline resulted in a first high-quality 3D reconstruction of the histological images, currently available at 100 μm. The dataset was further enriched by cortical surfaces and annotations. As part of the Julich Brain Atlas [6] 126 cortical and subcortical structures have been annotated in the histological sections with a resolution of 20 μm. The hippocampus [7] has been mapped in the 3D reconstructed data set.

References:

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