BigBrain: Initial Tissue Classification and Surface Extraction

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INTRODUCTION

Reference brains in human brain mapping are indispensable tools, enabling integration of multimodal data into a common framework. Previous atlases have not provided information beyond the macroscopic level. A notable challenge in approaching cellular resolution of the human brain is its sheer size and complexity, as well as the availability of imaging tools or computational technology capable of analyzing such an enormous dataset. However, fine-grain anatomical resolution of cortical layers, columns, microcircuits, and cells is necessary to fully understand neurobiological processes.

Pushing the limits of existing technology, in 2013 we published "BigBrain," a high-resolution 3D model of a human brain at nearly cellular resolution of 20 µm within- and between-planes, based on reconstruction of 7,404 histological sections [1]. This dataset is 125,000 times the resolution of a standard 1x1x1 mm^3 MRI.

In addition to our own group, several other research groups have begun to release high-resolution atlases, such as the Allen Human brain atlas [2] and patient H.M.'s brain atlas [3]; however, the 3D resolution of these atlases is highly anisotropic (i.e., due to thicker sectioning). To our knowledge, no other existing atlas has integrated 2D and 3D features of the entire brain in isotropic high resolution in order to provide a fully navigatable model.

Here, we extend our processing of BigBrain to include improved section-to-section intensity inhomogeneity correction, 3D tissue classification, and cortical surface extraction.

Histological processing inevitably introduces artifacts, which pose problems at all stages of the 3D reconstruction process. Defects include rips, folds, missing and displaced pieces, distortion, stain inhomogeneity, and crystallization (described previously in [1]). Differences in staining can occur due to, for example, the varying composition of the stain and exposition time of the section to air. Unlike MRI, for which the inhomogeneities form a continuous field, intensity inhomogeneities in histology are characterized by high variability in section-to-section staining density. As a prerequisite to cortical surface extraction, it was necessary to implement in-plane (2D) and across-section (3D) inhomogeneity correction.

METHODS

As previously described [1], we used a large-scale microtome to cut a paraffin-embedded brain (65-year-old female) coronally, acquired 7,404 sections at 20-µm thickness, stained for cell bodies applying Merker silver staining [4], and digitized each at 2,400 dpi in 16 bits, resulting in images of 13,000 by 11,000 pixels (10x10-$µm^2$ pixel size, ~1 TByte storage). A structural MRI was also collected prior to sectioning at 0.4x0.4x0.8 mm^3. Manual as well as automatic repairs were completed [5]; the repaired sections were registered to the MRI, which served as an undistorted frame of reference; further alignment was performed section-to-section with the use of nonlinear registration and the 3D volume was registered to stereotaxic MNI space (Fig. 1) [6].

In the current study, we first corrected in-plane (2D) staining artifacts via homogenization of the main tissue classes. Sampling points, defined on each section, were used for training of the tissue classification algorithm. Intensities for classified cortical gray matter were fitted with a 2D b-spline, then the section was normalized by this spline extrapolated to the entire section (white matter, brainstem, cerebellum).

In 3D, section-to-section intensity normalization was performed on the final non-linearly aligned sections, which had been resampled to a common field of view in order to obtain a contiguous volume in histological space. Section-to-section intensity imbalances were corrected by fitting a low-frequency b-spline to each coronal aligned section, then smoothing the b-spline coefficients (1,596) across the 7,404 sections at a FWHM of 5 mm. Each coronal section was normalized by the smoothed b-spline. The procedure was repeated over 5 iterations. Fig. 2 illustrates the inhomogeneities before (left) and after (right) section-to-section intensity normalization.

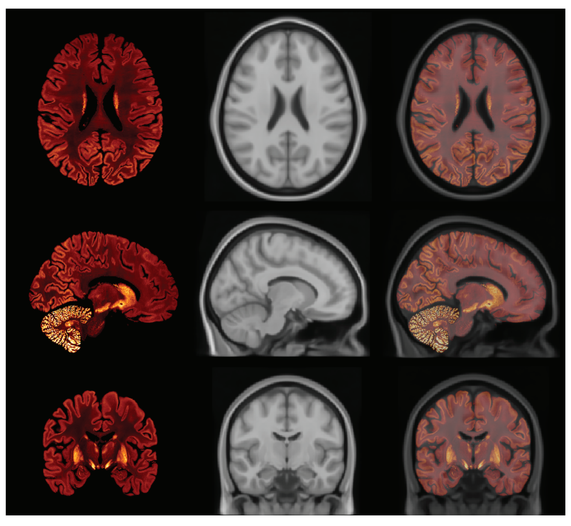

Fig. 1. BigBrain in MNI space. Left column, from top to bottom: Axial, sagittal, and coronal views of BigBrain down-sampled isotropically to 200 µm and registered to MNI space (500 µm). Middle column: MNI ICBM152 group average model [6]. Right column: Overlay of BigBrain and ICBM152 model in common MNI space.

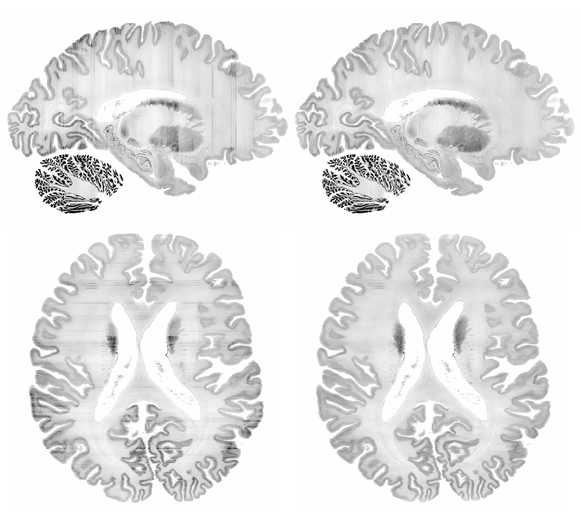

Fig. 2. Before (left column) and after (right column) section-to-section intensity normalization: 3D reconstructed views of BigBrain in histological space perpendicular to the sectioning direction, downsampled isotropically to 20 $µm^3$, visualized with Atelier3D navigation software [7].

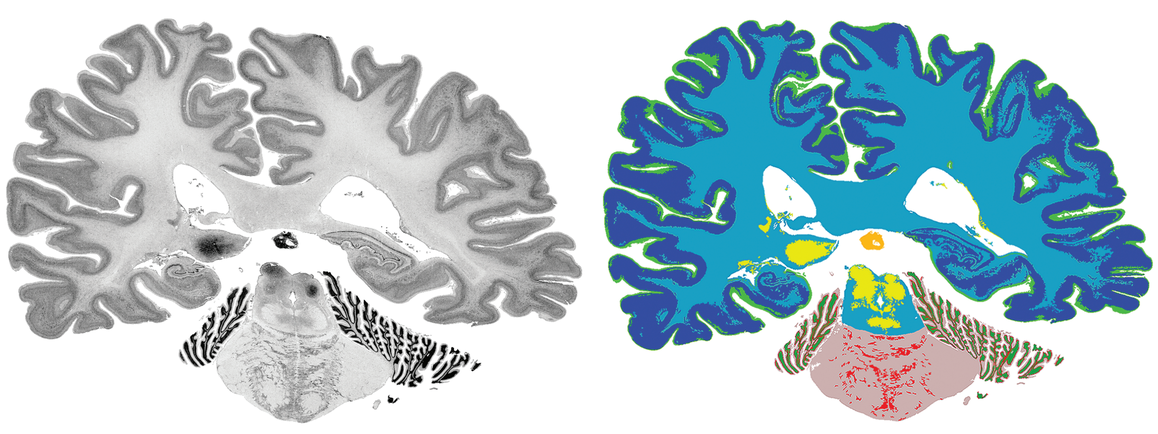

Fig. 3. Sample coronal section (20 $µm^3$) after intensity normalization (left) and subsequent tissue classification (right). Tissue classes include cortical layer 1 (light green), cortical gray matter (dark blue), cortical or brainstem white matter (light blue), subcortical or brainstem gray matter (yellow), pineal gland (orange), and cerebellum granular layer (dark green). Cortical layer 1 could be identified as a separate class (light green) by anatomically isolating voxels with white matter-like intensity outside of the gray matter mantle from those inside the gray matter mantle. In a similar way, deep gray matter regions (yellow) could be labeled distinctly from cortical gray matter (dark blue).

Next, the final tissue classification (2D, section-by-section) was performed in aligned space on the intensity-normalized sections in order to identify the major tissue classes (Fig. 3). A gross lobar segmentation was used to identify the white and gray matter in the cerebrum by hemisphere, the subcortical regions, the brainstem, and the cerebellum.

Segmented 3D volumes at 100, 200, 300, and 400 µm (isotropic) were reconstructed from the classified aligned coronal sections. The brainstem and cerebellum were masked off to retain only the cerebrum, which was split by hemisphere. Cortical surfaces were preliminarily extracted on the 400 µm volume using a marching-cubes algorithm from the CIVET pipeline [8] for the tessellation of the white matter and gray matter masks (Fig. 4).

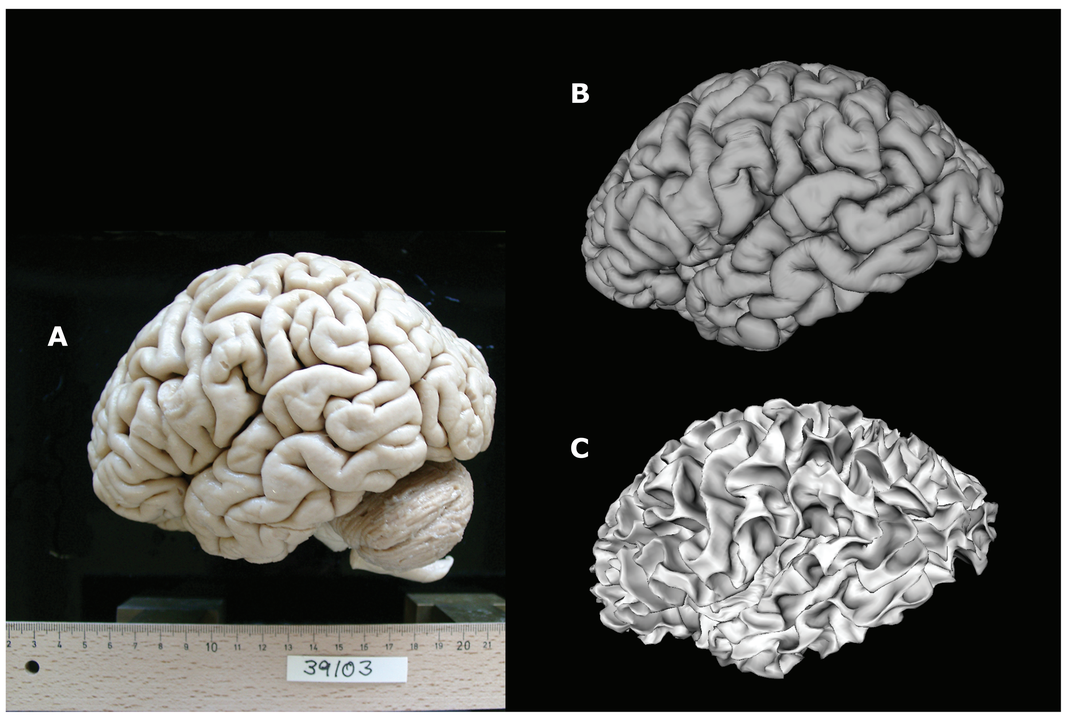

Fig. 4. BigBrain 3D surface models extracted at an isotropically-downsampled preliminary resolution of 400 µm. (A) Histological specimen prior to sectioning; (B) gray surface model extraction (163,842 vertices); (C) white surface model extraction (40,962 vertices). Some distortion in shape may have occurred due to handling.

RESULTS

The processing pipeline of BigBrain has been extended to include intensity inhomogeneity correction, tissue classification, and segmentation of the white and gray cortical surfaces at 400 µm (Figs. 4 and 5). The cortical surfaces are reported in a standard frame of reference via surface registration to the ICBM152 group average template [6, 9], thus enabling the use of surface parcellation atlases in MNI space.

BigBrain has previously been made freely available via the CBRAIN Portal (http://bigbrain.cbrain.mcgill.ca). We have updated the stacked 2D sections (intensity-corrected in 3D) at 20 µm in each plane and assembled 3D volumes in histological and stereotaxic space, which can be downloaded in MINC and NIfTI formats at 100, 200, 300, and 400 µm.

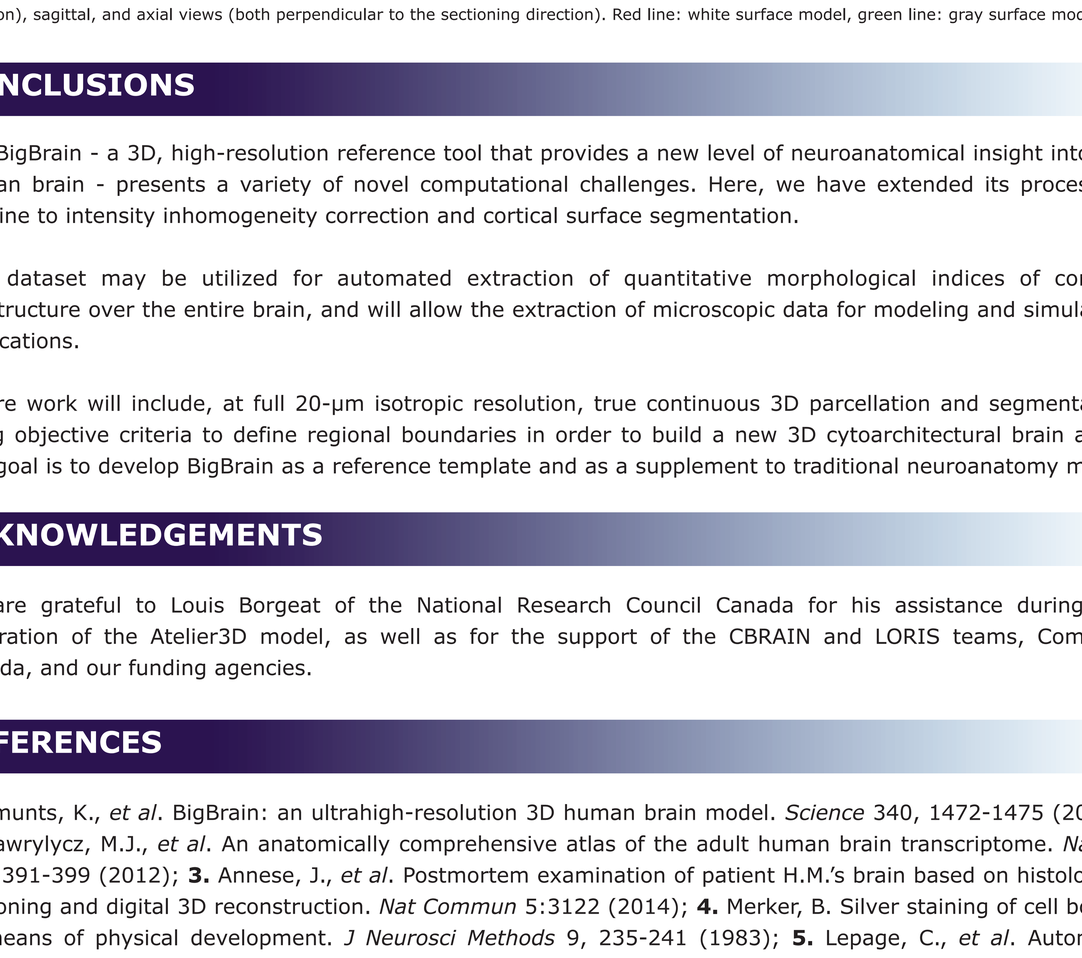

Fig. 5. Sample views of BigBrain surfaces (isotropically-downsampled to 400 µm resolution) overlaid on the model in histological space (isotropically-downsampled to 200 µm resolution). Top row, Broca's area; bottom row, Heschl's gyrus. From left to right: coronal (sectioning direction), sagittal, and axial views (both perpendicular to the sectioning direction). Red line: white surface model, green line: gray surface model.

CONCLUSIONS

The BigBrain - a 3D, high-resolution reference tool that provides a new level of neuroanatomical insight into the human brain - presents a variety of novel computational challenges. Here, we have extended its processing pipeline to intensity inhomogeneity correction and cortical surface segmentation.

This dataset may be utilized for automated extraction of quantitative morphological indices of cortical substructure over the entire brain, and will allow the extraction of microscopic data for modeling and simulation applications.

Future work will include, at full 20-µm isotropic resolution, true continuous 3D parcellation and segmentation using objective criteria to define regional boundaries in order to build a new 3D cytoarchitectural brain atlas. The goal is to develop BigBrain as a reference template and as a supplement to traditional neuroanatomy maps.

ACKNOWLEDGEMENTS

We are grateful to Louis Borgeat of the National Research Council Canada for his assistance during the generation of the Atelier3D model, as well as for the support of the CBRAIN and LORIS teams, Compute Canada, and our funding agencies.

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