Best Poster Award

A tomography slice through the entire human brain with less than three micrometer voxels

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ABSTRACT

Imaging anatomical features of the human brain at cellular resolution currently relies on series of physical sections with related slicing artefacts. So far, microtomography has been employed to image an entire human brain at a voxel size of 20 µm and selected regions using 6 µm. This study aims to demonstrate the feasibility of imaging the entire human brain with cellular resolution without the need for physical sectioning using hard X-ray computed tomography. 1.2 mm high sections of two human brains, one embedded in ethanol, the other in paraffin, were imaged using microtomography at the P07 beamline at DESY, Hamburg, Germany with a monochromatic beam at 67 keV. The extended field of view necessary to cover the *ca.* 10 cm wide specimens at 2.54 µm voxel size was realized by projection tiling with eight to ten rings. The resulting reconstructed slices measured 39,000 × 39,000 voxels. This synchrotron radiation-based study shows the feasibility of employing X-ray tomography to image the entire human brain with isotropic voxels of 2.54 µm resolution. Next, we need to tackle the vertical stitching of several 10,000 slices of 6 GB each, posing the challenge of processing the big data of an entire PB-sized human brain and making it accessible to the research community.

Keywords: Human brain, brain atlas, microtomography, mosaic acquisition, extended field-of-view, synchrotron radiation, cellular resolution

1. INTRODUCTION

Having access to cellular resolution volumetric data on the human brain is instrumental for understanding its structure and function in health and disease. The methods of choice for *in vivo* brain studies are based on magnetic resonance imaging (MRI), for example diffusion tensor imaging has been employed for obtaining connectivity mappings for studying stroke recovery.[1] However, even when used for *ex vivo* whole brain imaging, the spatial resolution is limited to tens of micrometers, not quite reaching true cellular resolution.[2] Histology using physical sectioning and staining remains the gold standard for *post mortem* brain imaging and forms the basis for current reference brain atlases. The reference atlas presented by Ding *et al.* consists of Nissl and immunohistochemistry stained sections spaced, respectively, 200 and 400 µm apart, with 1 µm pixelsize, aligned to MRI and diffusion weighted imaging volumes acquired at 200 and 900 µm isotropic voxel size.[3] The BigBrain model presented by Amunts *et al.* consists of a contiguous volume of 20 µm voxel size, produced from 20 µm thick sections stained for cell bodies, digitized at 10 µm in-plane pixel size and registered to an MRI reference.[4] While histological slices can be imaged at submicrometer resolution, the physical sectioning limits the resolution in the third dimension to tens of micrometers. Additionally the slicing introduces artefacts such as tears, folds,

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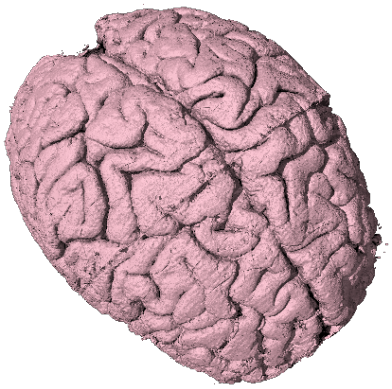

Figure 1. Volume rendering of a human brain scanned at 50 µm isotropic voxel size with a nanotom m (Waygate Technologies, Wunstorf, Germany) laboratory X-ray system in two height steps. The specimen was 140 mm long (posterior–anterior), 110 mm wide (left–right), and 80 mm high (inferior–superior).

or distortions, which need to be painstakingly removed. X-ray computed tomography gives access to three-dimensional structure in a non-destructive fashion and with isotropic spatial resolution. Phase contrast provides the enhanced density resolution necessary for soft tissue. The entire human brain has previously been measured using synchrotron radiation-based microtomography with a voxel size down to 20 µm.[5–7] For smaller specimens such as the mouse brain, cellular resolution volumetric data is available for the whole volume.[8] To achieve the same for the human brain, which is about 15 cm long and 12 cm in diameter, see Figure 1 for a volume rendering, poses new challenges for tomographic imaging. At 1.2 µm pixel size, a single projection image would need to measure 125,000×100,000 pixels to cover the entire brain. On the other hand the KIT CMOS detector used in this experiment featured 5120×3840 pixels at 6.4 µm pixel size. With 5× magnification, this gives an effective pixel size of 1.2 µm and a field of view (FOV) of 6.55×2.66 mm^2. To cover a much larger specimen, the FOV must be extended laterally and vertically. For this study only the lateral extension was addressed, and a projection tiling approach, as employed for example by Vescovi *et al.*,[9] was chosen. A series of scans with offset rotation axis were acquired and for each angle a projection covering the full width was produced, followed by a single reconstruction step.

2. MATERIALS AND METHODS

Two human brains were fixed in paraformaldehyde, dehydrated and stored in ethanol. One specimen remained in ethanol, while for the other coronal sections of 10 mm thickness were extracted and embedded in paraffin for X-ray imaging. Micro-computed tomography was performed at the P07 beamline at DESY, Hamburg, Germany with a monochromatic beam at a photon energy of 67 keV. The specimen was placed on the rotation stage 800 mm in front of the detector module, which consisted of a 100 µm thick $CdWO_4$ scintillator, mirror, 5× magnifying lens, and a 5120 × 3840 pixel KIT CMOS camera. The effective voxel size was 1.27 µm. The extended field of view was realized by the acquisition of eight rings for the paraffin-embedded specimen, and ten rings for the specimen in ethanol, each consisting of 48,000 projections over 360°. The exposure time was 30 ms. As the profile of the X-ray beam at P07 for this configuration was falling off rapidly in the vertical direction outside of a 1.2 mm high area, the actively used height of the detector was restricted to 951 instead of the full 3840 pixels, reducing the effective FOV vertically to 6.55×1.21 mm^2. For each angular step, the partial projections were aligned based on the recorded motor positions and blended to produce a 360° sinogram,[10] followed by tomographic reconstruction on 2 × 2-binned projections. The required acquisition times and data sizes are presented in Table 1. Ethical approval for this study was obtained from Ethikkommission Nordwest- und Zentralschweiz (EKNZ) under project no. 2020-00047.

Table 1. Acquisition time and storage requirements.

		Ethanol	Paraffin
One height step, 1.2 mm	Specimen diameter	11 cm	8.9 cm
	No. of rings	10	8
	Acquisition time	4.0 h	3.2 h
	Raw data size (uint16)	4.25 TB	3.40 TB
	Reconstructed size (binned 2 × 2, float32)	3.57 TB	2.39 TB
Projected values for full brain, 150 mm height	Acquisition time	20.8 d	16.7 d
	Raw data size (uint16)	531 TB	425 TB
	Reconstructed size (binned 2 × 2, float32)	446 TB	298 TB

3. RESULTS

Figures 2 and 3 show virtual coronal slices through the reconstructed volumes for the ethanol and paraffin embedded specimens, respectively. One slice of the paraffin dataset is 94.5 mm wide, corresponding to 37,207 voxels at 2.54 µm voxel size, *i.e.* after reconstruction from 2 × 2-binned projections. For the ethanol dataset the overall width is 115.4 mm, or 45,403 voxels at the same voxel size. The data can be enlarged at any position to examine features at the micrometer scale, *e.g.* details of the vasculature. Both datasets show signs of sample motion during the scan time of several hours. In the case of the paraffin specimen, the effect is amplified by the presence of air inclusions in the paraffin resulting from the preparation process.

4. DISCUSSION

Two sections of the human brain have been measured with 2.54 µm voxel size. Both were 1.2 mm high and about 10 cm wide, but had different embeddings, namely ethanol and paraffin. When extending the field of view in the vertical direction to cover the entire brain, this will be a substantial step in terms of spatial resolution compared to earlier volumetric images of the human brain. Imaging with isotropic resolution down to and beyond the cellular level has previously been demonstrated for the mouse brain[8] with a volume 3000 times smaller than the human brain. Here we demonstrated that the same extended field-of-view (FOV) imaging approach can be applied for the human brain. Instead of an eight times laterally extended FOV, an 20-fold extension is necessary, testing the limits of rotation axis alignment precision. The reconstructions shown here were obtained from transmission sinograms, without employing phase retrieval, as the phase retrieval step was observed to enhance the already present artefacts. More work is needed to mitigate this issue, as phase retrieval is central for improving soft tissue contrast, as illustrated for the cerebellum by Schulz *et al.* using grating interferometry.[11] The aforementioned artefacts are likely in parts due to sample motion during the three hours of scan time. Although the specimen was placed inside the beam hutch for acclimatization several hours prior to imaging, motion due to thermal expansion could still be a possible cause. The mitigation of sample motion needs to be carefully studied before imaging the entire brain.

Extending the imaged volume in the third dimension from the 1.2 mm high section presented here to the entire 150 mm length of the human brain will introduce challenges related to data handling and time requirements. Stitching reconstructed volumes vertically is straightforward in principle, but the size of these datasets will make the use of a dedicated multi-resolution registration pipeline[12] necessary. In the current configuration, more than two weeks of beamtime at a synchrotron radiation facility would be required for one brain dataset. Careful tuning of the monochromator could allow to reduce the exposure time further and potentially widen the beam in the vertical direction, which would both serve reducing the required beamtime. Projecting from the measurement of the ethanol-immersed specimen, the size of the processed whole brain dataset is estimated at over 400 TB. To make this available to the wider community requires a long-term hosting solution capable of this data volume. Downloading the entire dataset for local inspection will not be feasible for most researchers, therefore an online viewer for navigation across many length scales using a hierarchically organized data format should be considered, *e.g.* siibra-explorer[13] or Neuroglancer.[14]

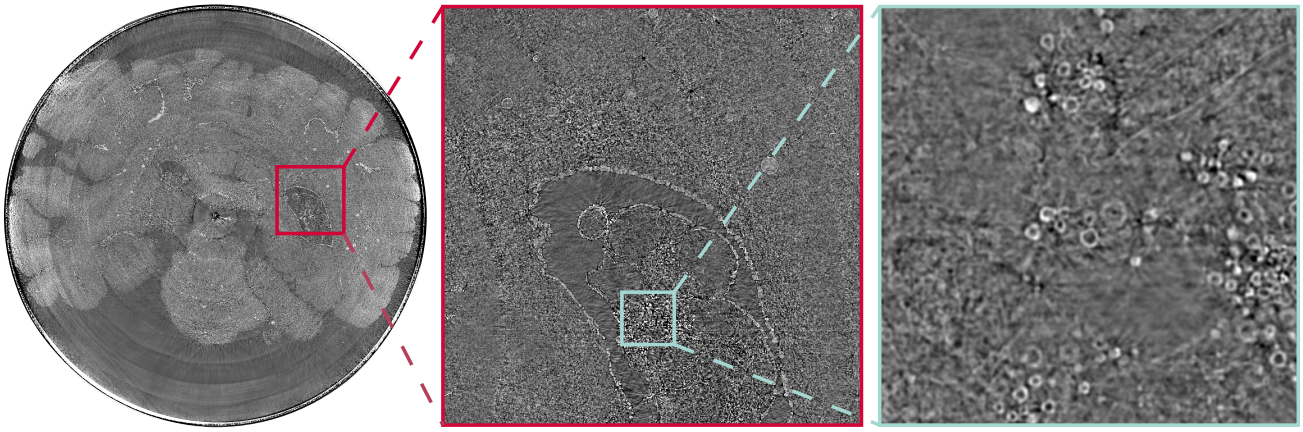

Figure 2. Virtual coronal slice through tomogram of ethanol-immersed brain with 2.54 µm voxel size. Left: A twenty times laterally extended field-of-view is required to cover the 11 cm diameter specimen. Center and right: Enlarged views of selected regions demonstrate the full resolution. The red box is 20.8 mm wide, the mint-colored one 2.6 mm.

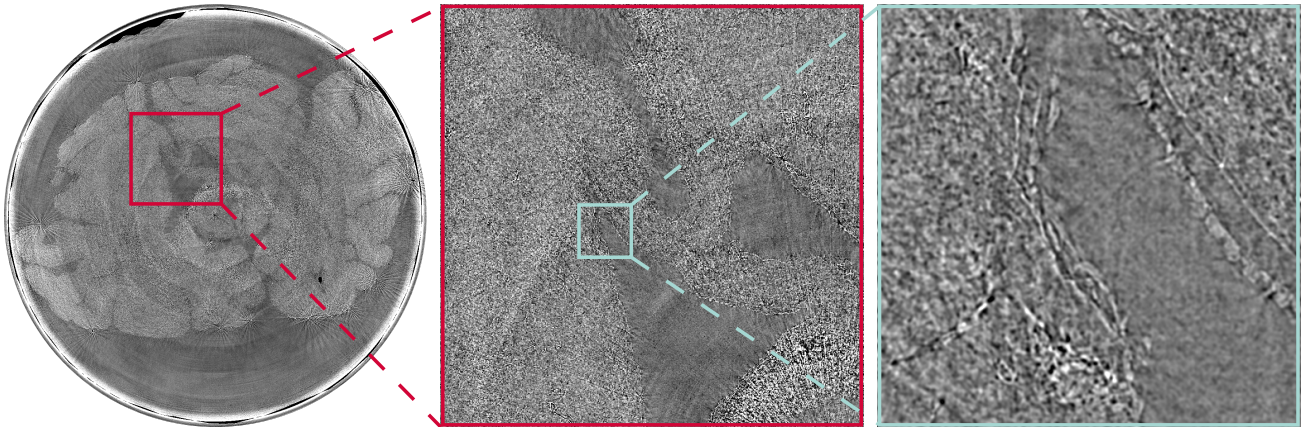

Figure 3. Virtual coronal slice as in Figure 2 for paraffin-embedded brain with a sixteen times laterally extended field-of-view to cover the 8.9 cm diameter specimen. The red and mint-colored boxes are 20.8 mm and 2.6 mm wide, respectively.

5. CONCLUSION

This study demonstrates the tomographic acquisition of one height step covering the entire width of a coronal section of the human brain with 2.54 µm voxel size. The acquisition strategy can be extended to allow imaging of the entire brain, by tiling projections not only horizontally, but also vertically. Making a multi hundred terabyte dataset available to the community in a form that can be interactively navigated will be a big challenge.

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