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Integrating histology and MRI in the first digital brain of common squirrel monkey, *Saimiri sciureus*

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Abstract

This effort is a continuation of development of a digital brain atlas of the common squirrel monkey, *Saimiri sciureus*, a New World monkey with functional and microstructural organization of central nervous system similar to that of humans. Here, we present the integration of histology with multi-modal magnetic resonance imaging (MRI) atlas constructed from the brain of an adult female squirrel monkey. The central concept of this work is to use block face photography to establish an intermediate common space in coordinate system which preserves the high resolution in-plane resolution of histology while enabling 3-D correspondence with MRI. *In vivo* MRI acquisitions include high resolution T2 structural imaging (300 μm isotropic) and low resolution diffusion tensor imaging (600 μm isotropic). *Ex vivo* MRI acquisitions include high resolution T2 structural imaging and high resolution diffusion tensor imaging (both 300 μm isotropic). Cortical regions were manually annotated on the co-registered volumes based on published histological sections in-plane. We describe mapping of histology and MRI based data of the common squirrel monkey and construction of a viewing tool that enable online viewing of these datasets. The previously described atlas MRI is used for its deformation to provide accurate conformation to the MRI, thus adding information at the histological level to the MRI volume. This paper presents the mapping of single 2D image slice in block face as a proof of concept and this can be extended to map the atlas space in 3D coordinate system as part of the future work and can be loaded to an XNAT system for further use.

Keywords

primate neuroanatomy; atlas; magnetic resonance imaging; diffusion tensor imaging

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1. INTRODUCTION

Atlases provide a central coordinate system in which to merge information from different imaging modalities, across time within a single subject, between subjects, and among studies. Digital atlases have come into common use from brain mapping with 3-D imaging techniques, e.g., magnetic resonance imaging (MRI). Integration of histology images with typical multi-modal MRI atlases (e.g., for the squirrel monkey [1]) is greatly complicated by the fact that histological sections are, by their nature, extraordinarily isotopic. Any out-of-plane rotation/resampling would result substantive partial volume artifacts.

During the sectioning process, it is commonly possible to acquire photographic images of the block face tissue that has yet been cut. Moreover, histology sections ($0.5 \times 0.5 \times 300 \mu\text{m}$) however occupy ~1GB per slice (and with ~500 slices per animal) and pose major space constraints for storage, processing and interoperability of this data. To resolve these issues, we turn to block face images, which are often acquired for quality control. Instead, we use block face imaging guides the reconstruction process with a spatial reference based on a volume formed by stacking digitized images of the surface of the remaining tissue block during histological sectioning [2]. Because the block is immobile, it maintains the spatial relationship between adjacent sections and serves as the ground-truth for the original position of histologic section.

In this paper, we advocate block face photography to establish an intermediate common space which preserves the high resolution. Also, defining the common space in coordinate system enables 3-D correspondence of histology data with MRI for easy comparison and faster access of data. We developed a viewing tool that enables online viewing of these datasets in different modalities.

2. METHODS

2.1 Data Acquisition

The data used for this topic is acquired as a part of larger project (Figure 1) to create a web based digital atlas of a squirrel monkey, *Saimiri sciureus*.

2.1.1 In vivo MRI Data Acquisition—Briefly, the brain of a healthy squirrel monkey (female) was imaged in three sessions on a 9.4 Tesla, 21cm bore Varian scanner (Varian Inc., Palo Alto, CA). The first two sessions were *in vivo* and had 20 days interval. During each *in vivo* session, the live squirrel monkey was anaesthetized and then scanned on its head with standard protocols of T2-weighted imaging (gradient echo multi-slice, TR = 404ms, TE = 2.4ms, flip angle = 20° , slice gap = 0mm, voxel size = $300 \times 300 \times 630 \mu\text{m}^3$, data matrix = $128 \times 128 \times 80$) and diffusion weighted imaging (pulsed gradient spin echo EPI, TR=5.5s, TE=44ms, number of gradient directions=20, $b \approx 1000 \text{s/mm}^2$, voxel size= $630 \times 630 \times 630 \mu\text{m}^3$, data matrix= $64 \times 64 \times 80$).

2.1.2 Ex vivo MRI Data Acquisition—A month later, under general anesthesia using aseptic techniques, a bidirectional tracer, biotinylated dextran amine (BDA; Molecular Probes Inc., Eugene, OR) was injected (as a 10% solution in phosphate buffer) into left

hemisphere M1. The monkey was allowed to recover from the procedure, giving the tracer time to be transported along axons to all regions connected to M1. One week after surgery, the monkey was given a lethal dose of barbiturate, and perfused through the heart. All blood was rinsed out with physiological saline (0.9% NaCl) followed by fixative (4% paraformaldehyde). The brain was removed from the skull and stored in buffered saline overnight. Then the brain was scanned as the third session (i.e., *ex vivo* session) including T2-weighted scans (gradient echo multi-slice, TR = 963ms, TE = 4ms, flip angle = 20°, slice gap = 0mm, voxel size = 300×300×300μm³, data matrix = 128×128×192, SNR ≈ 50) and diffusion weighted scans (pulsed gradient spin echo multi-shot spinwarp TR=4.6s, TE=42ms, number of gradient directions=31, b≈1000s/mm², voxel size=300×300×300μm³, data matrix=128×128×192, SNR ≈ 30) with full brain coverage.

2.1.3 Histologic Data Acquisition—After *ex vivo* scan, the entire brain was coronally sectioned to be a series of slices with 50 μm thickness each on a frozen microtome. The surface of the tissue block after each third sectioning was immediately photographed by a fixed camera. Photomicrographs of those Nissl-stained slides were obtained on a Leica SCN400 Brightfield Slide scanner at 20× magnification of the original size. It scans the whole slide at a low resolution, finds individual tissue sections at 20×, and then down samples 4 times for each brain section. Another three series adjacent to the Nissl-stained were stained for myelin, BDA and fluoro-ruby (see [3–5] for more details about the histological processing), Photomicrographs of those stained series will be collected the same way as the Nissl-stain and prepared for the development of histological atlas.

2.2 Data Processing

DTI data processing is performed in the coordinate system in which the data is acquired. In order to perform comparisons both within and between monkeys, it is necessary to align all data to a common space. From the non-diffusion weighted volumes of all datasets, a standard-space template was created using an iterative registration procedure similar to that used for atlas templates of the human brain. Next, the non-diffusion weighted volumes were registered to the template using linear and nonlinear registration procedures. By applying the resulting deformation fields, all tensor volumes, FA maps, MD maps, principle eigenvectors, and color maps were then transformed to the common space. The tensor, eigenvectors, and color maps were all re-oriented using the preservation of principle directions strategy.

All processing steps, including registrations, transformations, visualization, and file manipulations were performed with the MIPAV (Medical Image Processing, Analysis, and Visualization) software package and FSL FLIRT/FNIRT (FMRIB's Image Registration Tools).

2.2.1 Micrograph to DTI Space Registration—The digitized regions of interest (ROIs) were registered to the DTI image data using a multi-step registration procedure [3] which showed registration accuracy was approximately the size of one MRI voxel (0.3mm). First, every Nissl-stained section was down sampled and registered to the down sampled photograph of the corresponding tissue block using mutual information based 2D linear registration followed by a 2D nonlinear registration using the adaptive bases algorithm

(ABA). Next, all down-sampled block photographs were assembled into a 3D block volume, which was then registered to the *ex vivo* DTI volume using a 3D affine transformation followed by a 3D nonlinear registration using the Adaptive Bases Algorithm. The registration steps above produced deformation fields which were applied to the 2D digitized ROIs to transfer these regions into DTI space. Using the deformation fields described in “Data Processing” section, these ROIs were then aligned to the common space.

2.2.2 Histology Image to Block face Deformation—Histology images were captured in the Leica image file format. As documented in OpenSlide library [6, 7] and Open Microscopy Environment [8], Leica image files store a hierarchical series of tiles that form an image pyramid. In this way, users can access the data at a resolution appropriate for the task at hand instead of being forced to directly manipulate billions of pixels when only a few millions will be shown. First, a ROI was chosen on deformed image. Depending on the level of ROI, down-sample rate of that level was obtained from OpenSlide library [6] as indicated in Table 1.

Based on the coordinate of up-left corner point(x , y), width(w), height(h) of ROI, and down-sample rate(ds), we can get the corresponding original deformation field's up-left corner coordinate(x/ds , y/ds), width(w/ds), and height(h/ds). If corresponding original deformation field was $n*n$, then we grab $(n+1)*(n+1)$ for up-sample purpose. If $n*n$ region was on the right or bottom edge of the original deformation field, we set $n+1$ row or/and $n+1$ column to 0. The chosen deformation field then was up-sampled to the level of ROI based on down-sample rate. Next, from up-sampled deformation field, minimum and maximum values of x and y were found. Based on these values, a bounded image that contains all necessary coordinates for ROI was created. After that, we re-sample the up-sampled deformation field so that the up-sampled deformation field matches the coordinates of the bounded images. If we apply the generated deformation field to the bounded image, a ROI can be generated. There were two methods can be used while applying deformation field: nearest neighbor interpolation and bilinear interpolation. When nearest neighbor interpolation was chosen, all coordinates on deformation field would simply be rounded to the nearest integer. When bilinear interpolation was chosen, the program would find four pixels around every coordinates. Depending on the distances to the four pixels and the intensities of red, green, blue color channels at the four pixels, the intensities of R, G, and B color channels at those coordinates would be calculated. Now we set these RGB values to a new buffered image and a deformed image is created.

2.3 Slide Viewer Web Tool Construction

Our effort builds upon the foundation of OpenSlide [6] to read whole slide images (which can be billions of pixels, see Table 1), PanoJS3 [9] to provide tiled-viewing capabilities (and obviate the need to transmit the entire image from the server to the client), and custom code to overlay different data sources while deforming the images to match MRI and histology geometries.

As illustrated in Figure 2, we used PanoJS3 [9] on the front end for slide viewing and OpenSlide library on the back end on Tomcat server [10]. PanoJS3 is an interactive

JavaScript widget that allowing panning and zooming huge images which were stitched together from small tiles. It has a thumbnail image for preview. Since PanoJS3 viewer loads images tile by tile instead of whole slide image, it can be used for viewing images such as high resolution medical images that are much larger than the available space in the browser viewport. Depending on which part of images that users want to view, PanoJS3 calls the corresponding image URLs. The URLs contain images' information: level, x, y, image name, image path, and type of request (original image or deformed image). In order to use PanoJS3 to view the Leica format images, we create servlets to intercept PanoJS3's call for image URLs, read Leica images using OpenSlide library on Tomcat and save image tiles on the URLs. If the request is original image, we use OpenSlide library to read the image at specific location based on image path. Then, we select the requested section on the Leica image and save it on the URL. If the request is deformed image, we read the Leica image using OpenSlide library and load the Leica image's deformation field text file. Then we follow the above deformation method to up-sample and re-sample the deformation fields, generate bounded buffered images and interpolate them. We then save the deformed images on the URLs so that the slide viewer web tool will accept them. In the future version, we will make the viewer to call images and deformation field files that are saved on XNAT database and then we can view more images.

3. RESULTS AND DISCUSSION

We have constructed a model to integrate histology images with MRI in block face to address size constraints. We presented the design approach of choosing a common space coordinate system for mapping in block face and presented an example of BDA image that is tested across different browsers and different levels. Also we have developed a web tool to view the datasets and tested the approach across multi browsers and different levels as illustrated in Figures 3 and 4. As can be seen from the result section, we retain the detailed information that can be used for storage and analysis purpose without loading all data into memory or transferring data that would not be seen across a network. The ability to remotely navigate to the imaging data enables faster access of data without compromise in image quality. This tool also facilitates the quantitative comparisons across different imaging modalities.

In continuing work, we plan to extend this model to map the atlas space in 3D coordinate system. Meanwhile, we are developing a release platform that provides: (1) access to raw data in both native space and atlas space based on eXtensible Neuroimaging Archive Toolkit (XNAT, [11, 12]) and (2) custom portal for validation of DTI metrics (Figure 5). This atlas will provide the scientific community with a well-characterized collection of MRI and corresponding light microscopy data, which will allow investigators around the world to test various hypotheses related to the anatomy and function of the squirrel monkey brain in micro and macro scale. Making histological and MRI data freely available will facilitate many studies that one group, acting alone, would be unable to perform. For example, other potential applications include comparing connectivity patterns to other primates, parcellation of various sub-cortical gray matter structures, and validation of fiber tractography algorithms. This atlas will be useful to the neuroscience community by providing a tool to navigate, access, and visualize a wide range of neurological data.

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Figure 1.

Flowchart demonstrating the proposed data mapping design for integration of Histology with MRI using block face photography to establish an intermediate common space in coordinates without loss of resolution while addressing space constraint.

Figure 2.
System diagram representing software components of slide viewer tool

Figure 3.
BDA image as viewed from Slide Viewer web tool at different levels

Figure 4.
Deformed and Original Images viewed from different web browsers

Figure 5.
The XNAT portal provides by programmatic and end user access to all stages of processed data.

Table 1

Down sample rate, image size and resolution for level 0 to level 4

Level	Down sample rate	Width	Height	X ($\mu\text{m}/\text{pixel}$)	Y ($\mu\text{m}/\text{pixel}$)
0	1	64831	74816	0.5	0.5
1	4	16207	18704	2	2
2	16	4051	4676	8	8
3	64	1012	1169	32	32
4	256.1	253	292	128.05	128.05