Joint Atlas-Mapping of Multiple Histological Series combined with Multimodal MRI of Whole Marmoset Brains

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Abstract

Development of a mesoscale neural circuitry map of the common marmoset is an essential task due to the ideal characteristics of the marmoset as a model organism for neuroscience research. To facilitate this development there is a need for new computational tools to cross-register multi-modal data sets containing MRI volumes as well as multiple histological series, and to register the combined data set to a common reference atlas. We present a fully automatic pipeline for same-subject-MRI guided reconstruction of image volumes from a series of histological sections of different modalities, followed by diffeomorphic mapping to a reference atlas. We show registration results for Nissl, myelin, CTB, and fluorescent tracer images using a same-subject ex-vivo MRI as our reference and show that our method achieves accurate registration and eliminates artifactual warping that may be result from the absence of a reference MRI data set. Examination of the determinant of the local metric tensor of the diffeomorphic mapping between each subject’s ex-vivo MRI and resultant Nissl reconstruction allows an unprecedented local quantification of geometrical distortions resulting from the histological processing, showing a slight shrinkage, a median linear scale change of $\sim -1\%$ in going from the ex-vivo MRI to the tape-transfer generated histological image data.
1 INTRODUCTION

Marmosets are an increasingly important model species for both scientific and clinical neuroscience research. As compared with other non-human primate species, marmosets present certain advantages for brain mapping including a compact brain size and a relatively fold-free cortex, as well as the availability of transgenic capabilities [1], [2]. However, the formulation of a complete neural circuit map of a marmoset brain is a time consuming, mundane, and budget intensive process that requires multidisciplinary scientific knowledge and collaboration. One of the important sub-tasks in a circuit mapping project is to map whole-brain neuroanatomical data to a reference atlas. This is more challenging for a species like marmoset as compared to the laboratory mouse due to increased individual variation in brain geometry.

In this paper we focus on a specific methodology for obtaining brain-wide neurohistological data pertaining to brain connectivity mapping, where brains are sectioned into a series of thin sections each of which is subjected to a different histological procedure in order to obtain a full spectrum of information about cytoarchitectonic structure. The sections are gathered using a high-throughput neurohistological pipeline that has been previously described, together with multimodal in-vivo and ex-vivo MRI volumes in the same subject [3].

Guided histological reconstruction has been previously explored by several groups. The problem of accumulated long range distortions in unguided reconstruction was first posed by Malandain [4]. Block-matching was one of the earliest proposed guided-reconstruction solutions, using block-face photographs during histology to align sections [5]. Extracted features and manually defined landmarks have also been applied to guide reconstruction [6]. More recent methods have used same-subject MRI to guide reconstruction of human [7] and mouse [8] brain structures.

Here, we propose an automatic computational pipeline for same-subject MRI reference guided reconstruction of multi-modal histological image stacks followed by registration to a labeled atlas. Our reconstruction process is informed by ex-vivo MRI of the brain prior to histology as well as an image intensity smoothness prior. To perform atlas-mapping, we use a variant of the multichannel large deformation diffeomorphic metric mapping (LDDMM) algorithm which applies voxel-level weights to the image similarity metric to account for damage or noise in histological sections. We apply our method to a dataset of marmoset brains obtained as part of the Japanese Brain/MINDS project and show reconstruction and segmentation results across four modalities, as well as high-resolution tract tracing results. Finally, we perform a quantitative analysis of the distortion caused by the histology process by
comparing the ex-vivo MRI with Nissl reconstructions.

2 RESULTS

2.1 Algorithm workflow

Accurate reconstructions of histological volumes that avoid the classical error of accumulated curvature distortion can be achieved by guiding the reconstruction process with a shape prior. Marmoset brain histology protocols generally include ex-vivo MRI of the unsectioned brain, thus, a same-subject reference volume is available and the shape prior can be considered near-exact up to a global 3D affine transformation and imaging noise. After guided reconstruction, the subject brain is mapped to the Brain/MINDS atlas \[9\], a 3D digital labeled whole marmoset brain atlas with Nissl-stained and MRI volumes. Cross modality registration is performed using the Nissl reconstruction as an exact shape prior. Figure 1 illustrates this workflow.

Figure 1: Reconstruction and registration pipeline workflow from multimodality histological image sections to segmented data and connectivity analysis.

2.2 Validation in simulated data

Simulated data was generated to evaluate the accuracy and consistency of the pipeline. In each simulation, the Brain/MINDS Nissl-stained volume was re-sectioned by applying random rigid motions to each coronal section of the volume. The random rigid motions were Gaussian-distributed, centered at...
zero, with a standard deviation of 20 pixels (1.6 mm) for translation and 25 degrees for rotation. A random affine transformation was applied to the corresponding Brain/MINDS MRI volume. The simulation was performed in one case with Gaussian white noise (zero-centered with standard deviation of half the mean intensity value of the image) applied to distort the MRI, and another case with no noise. The simulated data is passed through the guided reconstruction pipeline and the error, variance, and bias of the reconstruction parameters are compared with the ground truth parameters. The simulation was repeated 1000 times. The simulation results are summarized in Figure 2.

Figure 2: RMSE, standard deviation, and norm of bias statistics for estimated MRI-guided reconstruction parameters from simulated Brain/MINDS phantom.
2.3 Application to Brain/MINDS marmoset histology data

The proposed pipeline was applied to a set of twelve marmoset brain histology image sets which were alternately Nissl-stained for structural information and fluorescence-imaged to perform tract-tracing from trans-synaptic viral tracer injections. The data is originally acquired at 0.46 micron in-plane resolution and is downsampled to 80 micron in-plane resolution for registration. Figure 3 depicts an example of a subject for which unguided reconstruction by minimizing error between neighboring sections failed to produce a realistic reconstruction, followed by correction by MRI-guided reconstruction.

![Unguided vs MRI-guided reconstruction](image)

Figure 3: Comparison of unguided versus MRI-guided Nissl stack reconstruction in Brain/MINDS marmoset dataset. The unguided reconstruction (left) is a simple minimization of squared error from section to section and shows significant distortion along the sectioning axis. The MRI-guided reconstruction (right) produces a shape similar to that of the reference MRI (middle) while maintaining coherence and continuity from section to section.

A variant of the large deformation diffeomorphic metric mapping (LDDMM) algorithm that accounts for missing structures or damaged sections in the subject image was employed to compute nonlinear maps between the Brain/MINDS Nissl atlas and the reconstructed Nissl subject. The atlas parcellations were then cast onto each subject, as depicted in Figure 4.

In order to segment the fluorescence data, the same reconstruction framework was applied to the un-reconstructed fluorescence image volume, using the corrected Nissl stack as an exact shape prior with no global affine transformation obfuscation between Nissl and fluoro. Figure 4 shows an example of a fluoro image stack being reconstructed and assuming the segmentation computed on the structural Nissl stack.
Figure 4: Sample segmentation of Nissl and fluorescent reconstructed volumes. The upsampled reconstruction transforms are applied to the full resolution fluorescent tracer images where tract tracing can be performed. Here, the three injected tracers are labeled in the high resolution image (bottom left).

The computed transforms are applied to the full resolution fluorescence image stack, where tract tracing can be performed (Figure 4, bottom panel). Major connections and fiber tracts can be identified from the color-coded stains in 3D. A visualization of these 3D tracings can be found in the supplementary video, several frames of which are displayed in Figure 5.
Figure 5: 3D visualization of the Nissl stack reconstruction overlayed with the red, green, and blue tagged viral tracer paths detected from the registered fluorescence volume.
2.4 Analysis of shape change induced by sectioning process

In addition to atlas mapping, each subject MRI was diffeomorphically mapped to its corresponding reconstructed Nissl stack using a variant of the LDDMM algorithm with a mutual information similarity metric. Up to this point, the pipeline only computed affine transforms between the MRI and Nissl stacks. The diffeomorphic mappings computed here combined with the scale/shear factors from the affine transforms encapsulate the non-rigid deformations of the subject brain induced by the histology procedure, and the Jacobian determinant of these mappings can be used to analyze change in shape. Figure 6 displays the corresponding exvivo MRI-to-Nissl transformation as a percentage of local scale change (by computing the cube root of the local Jacobian determinant) for the central sagittal section of each subject, alongside histograms of the scale change for each entire brain volume.
Figure 6: Comparison of deformation caused by the histology procedure in each of 12 subjects from Brain/MINDS dataset. The subject’s ex-vivo MRI is overlayed with the percent scale change, where blue represents shrinkage and red represents expansion. A histogram of the percent scale change per voxel is plotted alongside its corresponding brain.

The twelve brains were pooled by mapping their Jacobian determinants to the space of the Brain/MINDS atlas using the computed mappings presented previously. The mean percentage of local scale change at each voxel of a central sagittal section is plotted in Figure 7.
3 DISCUSSION

As first described by Malandain in the context of brain histology [4], correct reconstruction of a sectioned object without prior knowledge of the object’s shape is difficult and easily prone to error. Here, we inform our histological reconstruction algorithm with the best available shape prior – either an MRI of the brain prior to sectioning or an atlas volume of the same species and modality. We show in simulation that same-subject reference guided reconstruction is able to recover the resectioning parameters with high accuracy, which not only provides values in improved visualization but also improves registration and segmentation accuracy.

When the shape prior is a same subject reference volume, the guided reconstruction acts as an improved initialization for nonlinear image registration, placing the voxels of the subject volume closer to their corresponding voxels in the atlas volume. As with any gradient-based optimization framework, LDDMM benefits from improved initialization as this reduces the likelihood of falling into a local minima in the objective function. Figure 8 depicts sample maps generated by registration of the Brain/MINDS atlas to an unguided reconstruction versus a guided reconstruction. The increased curvature of the gridlines in the unguided case indicates a displacement field with higher magnitudes and less homogeneity.
Figure 8: Comparison of nonlinear maps produced by registering the Brain/MINDS atlas to an unguided Nissl stack reconstruction versus an MRI guided Nissl stack reconstruction. Top row shows unguided (left) and guided (right) subject Nissl reconstructions. Bottom row shows corresponding nonlinear maps produced by LDDMM optimization.

The addition of the smoothness prior is valuable for providing robustness in the presence of noise or missing data or when the shape prior is not an exact reference volume. The driving intuition behind the smoothness prior is that in addition to the subject brain taking the shape of the reference volume, its image should be continuous and smooth. The effect of this prior is particularly noticeable in the registration of multiple subject modalities to one another where sections are missing or damaged. This is visible in the top row of Figure 9.
Figure 9: Reconstruction examples depicting the effect of the smoothness prior. When the Nissl stack (top right) has missing or noisy sections, reconstruction to the next-best Nissl section contains small distortion accumulations (top left). Inclusion of a smoothness constraint corrects this error (top middle). Additionally, highly damaged Nissl stacks can still be reconstructed despite major differences between damaged Nissl sections to corresponding MRI sections.

The effect of the image intensity smoothness prior also manifests during Nissl-to-MRI stack reconstruction when there is significant damage to the Nissl brain. The bottom row of Figure 9 shows that we are able to achieve accurate reconstruction even when this is the case.

Inspection of Figure 6 reveals that the general deformative effect caused by the histological process is shrinkage in certain areas of the brain. This effect is not surprising as it is generally well-known that some tissue shrinkage is caused by the histology procedures [10]. Examination of the mean image (Figure 7) shows that shrinkage is generally located in a central inferior region of the brain, and near the ventricles.
4 METHODS

4.1 Imaging and tissue preparation

All experimental procedures were approved by the Institutional Animal Care and Use Committee at RIKEN and conducted in accordance with the Guidelines for Conducting Animal Experiments at the RIKEN Center for Brain Science. Common marmosets (Callithrix jacchus), 4 to 8 years old, 330g - 440g in weight, were acquired from the Japanese Central Institute for Experimental Animals (CIEA). MRI scans were performed using a 9.4-T BioSpec 94/30 US/R MRI scanner (Bruker, Ettlingen, Germany) with actively shielded gradients that had a maximum strength of 200 mT/m. Individual marmoset is being placed with four tracers in the right hemisphere, two anterograde tracers: AAVTRE3TdTOM (AAVdTOM) and AAVTRE3Clover (AAVGFP); two retrograde tracers: FastBlue (FB) and CTB for reasons of parsimony. High resolution T2-weighted images (T2WI) were acquired using a rapid acquisition with relaxation enhancement sequence to preserve a true shape prior after perfusion. Serial sections cut in the coronal plane with consistent high-quality tissue were put through the histological pipeline, which was optimized for high-throughput processing.

Based on the individual marmoset brain volume (distance of AP) measured by ex-vivo MRI, the number of sections (20 µm/section) can be cut into 1600-1700 sections. We employed a modified tape-transfer assisted cryosectioning technique to preserve the geometry of individual sections [11]. Separate histological processes based on the series’ intended use (Nissl, myelin, fluorescence, CTB) is applied. Each series has 80 µm section spacing. Imaging data were collected from the Nanozoomer 2.0HT and then automatically transferred to processing server, onto the data-acquisition server. All scanned images were acquired using a 20x objective (0.46 µm/pixel in plane) and saved in an uncompressed RAW and JP2000 format. Nissl, myelin and CTB stained slides used brightfield scanning. Fluorescent images used a tri-pass filter cube (FITC/TX-RED/DAPI) at 12-bit depth per pixel/color channel for anterograde tracers AAVdTOM and AAVGFP and retrograde tracer FB injections. All data were subsequently processed in a storage cluster and were subjected to lossless compression for storage and lossy compression for further data analysis. Figure 10 shows the flowchart of the entire histological processes (injection, MRI, perfusion, embedding, sectioning and staining) to data acquisition processes (imaging, cropping, conversion, quality control and registration) [3].
4.2 Same-subject reference guided stack reconstruction

Define the observed subject histological stack $J_i$ as an obfuscation of a ground truth reconstruction $J_0$ by a series of two-dimensional in-plane rigid motions $R_i^{-1}$.

$$J(x, y, z_i) = J_0(x, y, z_i) \circ R_i^{-1}(x, y, z_i)$$

(1)

As $J_0$ is unobservable, our aim is to recover $R_i$ and produce an estimate of $J_0$. We employ a reference volume of the same subject, $G$ (for instance, a pre-histology MRI) and consider it to be an affine transformation of $J_0$.

**Intensity Prior:** Following the method described in [12], we impose a prior on the smoothness of $J$ under the natural assumption that anatomical structures are smooth and continuous. We write the image $J$ as arising from a smooth Sobolev space $H^k$ equipped with norm:

$$||J||_{H^k}^2 = \sum_k \int_{\mathbb{R}^3} |\partial_h J(\cdot, z)|^2 d\cdot dz$$

(2)

We also define the discrete derivative operator $D_h$ which operates along $z$, the sectioning axis:

$$D_h f(x, y, z) = \partial_{h_1, h_2} \left( \frac{f(x, y, z + \Delta/2) - f(x, y, z - \Delta/2)}{\Delta} \right).$$

(3)
Shape Prior: We additionally impose a prior on the shape of the reconstructed stack via the same-subject reference. The relation between the stack reconstruction and the reference image is defined as:

\[ J(x, y, z_i) \circ R_i(x, y) = G(x, y, z) \circ A(x, y, z) \]  

Where \( A \) is an unknown affine transformation.

Combined Likelihood: Combining the priors and regularizing the estimated transformation parameters \( R_i \) and \( A \), we obtain the combined log likelihood which serves as our objective function to be minimized.

\[ \arg\min_{R_i, A} \frac{1}{2} \sum_i ||D_h J \circ R_i||_2^2 + M(J \circ R_i - G \circ A) + \log \pi(R_i) + \log \pi(A) \]  

Where \( M \) is an image similarity metric and \( \pi \) indicates a regularization on the parameters to be optimized. In cases where the reference volume and target stack are imaged by the same modality, sum of squared error can be used, such that \( M(J, I, R_i, A) = ||J \circ R_i - G \circ A||^2 \). In cases where the reference volume is acquired from a different modality, the mutual information cost metric is employed.

4.3 Diffeomorphic atlas-mapping

The Brain/MINDS Nissl marmoset brain atlas is nonlinearly mapped to the reconstructed subject Nissl stack (after an initial affine alignment) in order to produce segmentations of the subject imagery. We employ a multi-channel, cost-masked variant of the large deformation diffeomorphic metric mapping (LDDMM) algorithm to compute nonlinear volume registration of the atlas \( I \) to the reconstruction \( J^R \) using sum of squared differences as the image similarity metric. The objective function to be minimized takes the form:

\[ \hat{v} = \arg\min_v \int_0^1 ||v_t||_V^2 dt + \sum_{n=1}^N \frac{1}{\sigma_n^2} ||C_n(I_n \circ \varphi^{-1} - J_{R_n}^R)||_{L^2}^2 \]  

The optimization is performed over the parameter \( v_t \), a time varying velocity field which parameterizes a diffeomorphism, \( \varphi_t \). The diffeomorphism is generated by integrating \( v_t \), solving the ordinary differential equation:

\[ \dot{\varphi}_t = v_t \circ \varphi_t, \; t \in [0, 1], \; \varphi_0 = \text{Id} \]
We specify a reproducing kernel Hilbert space $V$ equipped with norm $\| \cdot \|_V$, and we take the norm of the velocity field $V_t$ as a regularizer on the smoothness of the computed mapping.

Here, the data attachment term (2nd term of Eqn. [6]) is a sum over $N$ image channels, where we generally set the normalized atlas and subject images as the first channel and masks of automatically segmented features such as the brain boundary or ventricles as following channels. The weight of each channel in the similarity metric is controlled by the parameter $\sigma_n$. We accommodate for missing/damaged sections or structures by a mask $C_n$ on the similarity metric in the subject coordinate space. Here, $C_n$ is a matrix of the same size and dimension as $I$ and $J^R$ taking on values from 0 to 1, where 1 represents full confidence in the subject data, 0 represents no confidence in the subject data (i.e. histological sections that are known to be excluded), and values in between indicate varying noise levels (i.e. partially damaged histological sections). This cost mask is multiplied element-wise with the similarity metric.

### 4.4 Cross-modality registration

In histological imaging, the structural image stack (here, the Nissl stack) is often not the data of interest. The Brain/MINDS dataset also acquires fluorescence viral tracer, immunohistochemical, and myelin imagery, which contain information about connectivity, and each of which must be segmented using the same atlas parcellations. To achieve this end goal, we apply the method described above in section 4.2 with a modification to the shape prior:

$$J(x, y, z_i) \circ R_i(x, y) = G(x, y, z) \quad (8)$$

We set the reconstructed Nissl stack as the same-subject reference volume $G$ and assuming $A = \text{Id}$. The objective function then takes the form:

$$\hat{R}_i = \arg\min_{R_i} \frac{1}{2} \sum_i \| D_h J \circ R_i \|_2^2 + M(J \circ R_i - G) + \log \pi(R_i) \quad (9)$$

Where $M$ is the mutual information image similarity metric.

### 4.5 Shape analysis

To assess the deformative effects of the histological sectioning process on brain tissue, we compared the shape of the ex-vivo pre-sectioning MRI with the reconstructed Nissl stack in a dataset of 12 brains. Each subject’s MRI was registered to its corresponding Nissl reconstruction using single-channel
LDDMM with a mutual information similarity metric \cite{13}. The resulting transforms were compared across subjects by examining the cube root of the determinant of their local Jacobians at each image voxel. We call this measure the local scale change, where $J_i$ is the local Jacobian determinant at particular control point:

$$\text{scale change} = J_i^{\frac{1}{3}}$$

(10)

These were plotted as percentage of local scale change, alongside histograms of this data, where histograms pooled values at voxels which were considered part of the brain tissue.

Each subject’s Jacobian volume was also mapped back to the space of the Brain/MINDS atlas such that every voxel contains local scale change at corresponding voxels across subjects. The mean of the pooled scale change was computed.

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Competing Interests

M.I.M. reports personal fees from AnatomyWorks, LLC, outside the submitted work and jointly owns AnatomyWorks. This arrangement is being managed by the Johns Hopkins University in accordance with its conflict of interest policies. M.I.M.’s relationship with AnatomyWorks is being handled under full disclosure by the Johns Hopkins University.

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