



Software tools for reconstruction of 3D projection patterns for injection-based tractography

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Mouse Brain
Architecture

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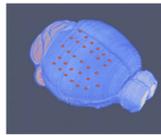
Overview

Objective

Develop software tools that support automatic data processing, including quantitative analysis and 3-D visualization, of the large volume of neuroanatomical tractographic data generated by modern digital imaging of whole brain data sets (as, e.g., our Mouse Brain Architecture (MBA) project; <http://mouse.brainarchitecture.org>).

Data

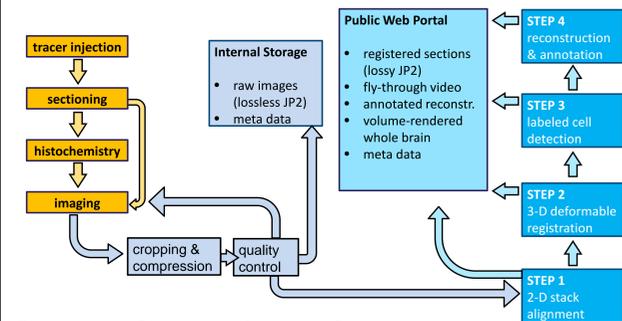
Tracer-injected, thin-sectioned, whole brains; tracer series scanned with 0.5 $\mu\text{m}/\text{pixel}$ in-plane resolution. Injection sites (each in a different animal) are sampled on a 3D grid covering the entire brain.



Tracer	Type	Label	Images
biotinylated dextran amine (10kD; 10%)(BDA)	classical anterograde	IHC-amplified density	
cholera toxin subunit B (1%) (CTB)	classical retrograde	IHC-amplified density	
adeno-associated virus AAV2/1.CB7.C1.EGFP.WPRE.RBG AAV2/1.CAG.tdTomato.WPRE.SV40 (AAV)	viral anterograde	GFP/ RFP in axons	
modified rabies virus RV-4GFP(B19G) (RV)	viral retrograde	Cytoplasmic GFP in somata	

MBA Pipeline:

Histological Processing \rightarrow Image Processing & Analysis



Principal Steps of Image Analysis

Step 1. 2-D Stack Alignment

Create brain stack from section images aligned within a common reference frame via 2D rigid transformations (translation and rotation). Nissl stack created first; alternating sections developed for tracer are inserted and aligned next.

Step 2. 3-D Deformable Registration

The brain stack is registered to a volumetric reference atlas by optimizing correspondences between the volumes through a nonlinear transformation.

Step 3. Detection of Labeled Cells

Automatically detect labeled features of interest (i.e., retrogradely labeled somata or anterogradely labeled axonal fibers and terminations).

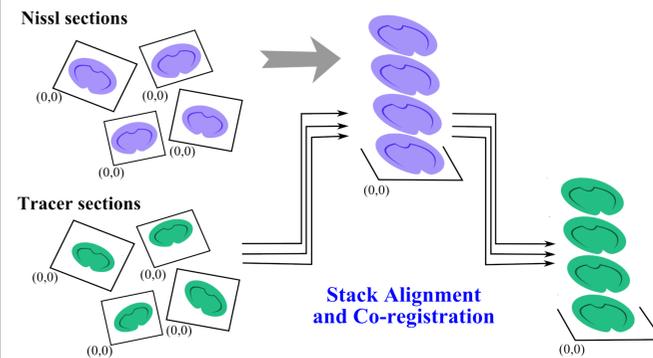
Step 4. Reconstruction & Annotation of Projections

Reconstruct neuronal projections by algorithmically linking and annotating automatically detected histological features in reference frame.

Step 1. 2-D Stack Alignment

Summary

- Nissl images show stained cell bodies; their position collectively carry structural information about the brain.
- Alternate sections are developed for Nissl and tracer
- Adjacent (Nissl and tracer) sections have similar shape
- Alignment algorithm has two step (scheme shown below)
- Step 1: Alignment of the Nissl image stack
- Step 2: Co-registration of each image in tracer series with adjacent Nissl images.

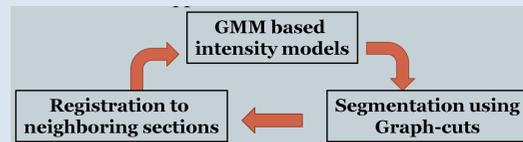


Alignment algorithm for the Nissl images

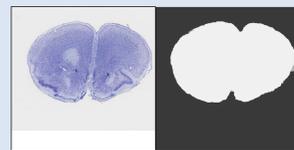
- iteratively estimate the tissue mask and the transformation with the adjacent Nissl image.
- Estimate the parameters of a centered rigid transformation model for each Nissl section 's optimal alignment with the Nissl stack.
- In the iterative multi-scale optimization (shown below) segmentation and registration mutually benefit
- segmentation is informed by the shape of adjacent sections
- registration improves when applied to segmentation mask

$$Y_i - c_i = R_i (X_i - c_i) + t_i$$

where
 Y_i, X_i : Image coordinates after and before transformation
 c_i : Center of rotation
 t_i : Translation
 R_i : Rotation matrix

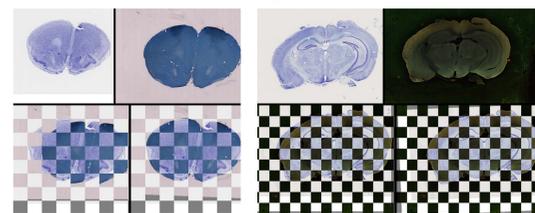


- Illustration of the tissue mask estimated using the above scheme.



Co-registration of Tracer and Nissl images

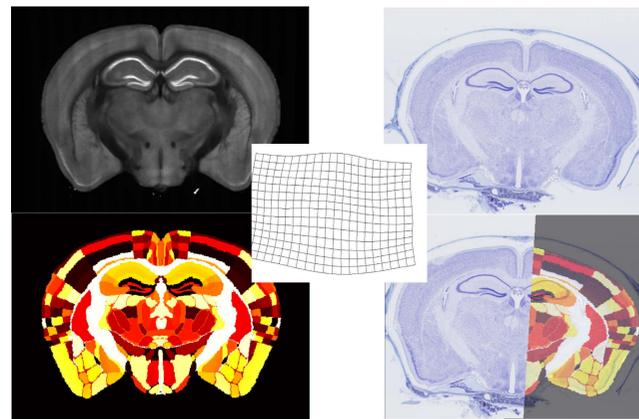
- Each image in the alternate tracer series is co-registered to its Nissl neighbor during the co-registration step.
- The multi-scale optimizer maximizes the mutual information between the Nissl-tracer image pairs.



Step 2. 3-D Deformable Registration

Multimodal deformable registration^[1]

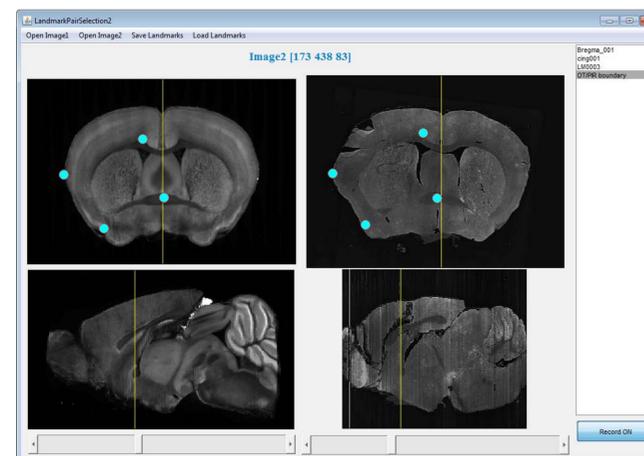
- Step 1: Affine transformation
 - Step 2: Free-form deformation estimated on a 3D grid
 - 3D Grid-size: 240 μm cube
 - Resampling: cubic-splines
 - Multi resolution: 5 levels
 - Objective function: max. normalized mutual information
- [1] S. Klein, M. Staring, K. Murphy, M.A. Viergever, J.P.W. Pluim, "elastix: a toolbox for intensity based medical image registration," IEEE Transactions on Medical Imaging, vol. 29, no. 1, pp. 196 - 205, Jan 2010.



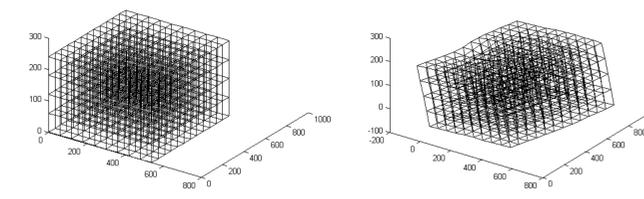
Deformable registration for Mapping between spaces and modalities

Deformable registration aided by anatomical landmarks – under construction

- The landmark warping tool uses thin-plate splines to estimate the optimal non-linear transformation between the corresponding landmarks of target and reference brains.
- The graphical user interface allows rapid inspection, editing and labeling of anatomical landmarks.



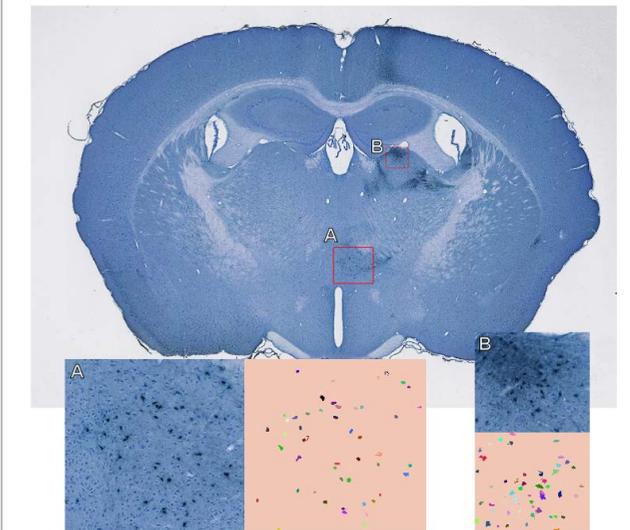
- Deformation field visualized on a deformed mesh.
- Effort is directed towards development of automated detection and matching of landmarks.



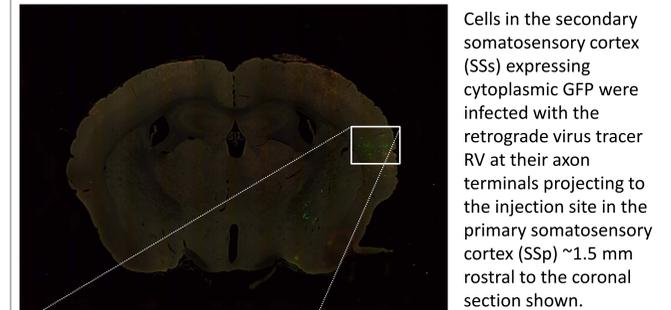
Step 3. Detection of Labeled Cells

Summary

- cell detection achieved using graph based segmentation
- process detection achieved using contrast enhancement for elongated structures, followed by gray-scale skeletonization



Cells detected with somatic CTB label in nuclei of the dorsal thalamus, (A) LD and (B) RE. These retrogradely labeled neurons project to the injection site in association cortex (PTLp) ~ 1 mm caudal to this coronal section.



Cells in the secondary somatosensory cortex (SSs) expressing cytoplasmic GFP were infected with the retrograde virus tracer RV at their axon terminals projecting to the injection site in the primary somatosensory cortex (SSp) ~ 1.5 mm rostral to the coronal section shown.

Continued Work

The reconstruction step (Step 4. in our Analysis work-flow) is under development. This step reconstructs neuroanatomical connectivity by algorithmically linking and annotating automatically detected histological features in the reference frame.

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