Brain-wide analysis of rodent brains using standardized mouse and rat reference atlases

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Abstract

Molecular neuroanatomical methods have expanded the ability to map connections and activity of neuron subtypes and circuits in whole-brain experimental models. When performed on mouse and rat brains that have been registered to a reference atlas, these analyses reveal details about the functional organization of brain circuits related to behavior and pathologies that are comparable across animals, experiments, and laboratories.

Here we present advances to our research on reconstructing whole slide images of mouse and rat brains from sections labeled with histochemical techniques into whole-brain 3D image volumes with anatomic constrains imposed by the Allen Mouse Brain Common Coordinate Framework (CCF, for mouse), or the Waxholm Rat Brain Atlas (for rat). This advancement results in a compiled section brain image volume that is closer in shape to the in vivo brain. Once images are registered to a reference space, cellular populations and expression intensities (e.g. for cFos) are quantified for each brain region.

We also present mapping tools for registering intact brain volumes imaged with light sheet microscopy. Intact mouse or rat brain volumes imaged from cleared brain tissues by light sheet microscopy methods can be registered to the Allen CCF or Waxholm Rat Brain Atlas using linear and nonlinear registration methods.

We also present a neuroscience research study of whole brain analysis of connectivity using trans-synaptic rabies labeling of neurons. For this analysis, whole slide images of coronal brain sections were reconstructed into a 3D volume, labeled neurons were automatically marked using a neural network, and brain sections and detected neurons were registered to the CCF. The number of labeled neurons in each of the 2500 brain structures in the CCF were calculated, allowing for comparative quantitative analysis between mice. Similar results are presented for rat brain volumes expressing cFos. We also present the results of registering mouse brain volumes from light sheet imaging to the CCF.

Methods

Fluorescent specimen compilation

Mouse brains comprised of over one-hundred coronal sections from mice injected with an AAV-Cre-dependent expression vector and counterstained with fluorescent Nissl stain were imaged by fluorescent whole slide imaging using Neurolucida. Sections from these images were automatically extracted, aligned, and compiled into full resolution 3D whole-brain images using BrainMaker and are available as part of the GENSATcreBrains project (GENSATcreBrains at Biolucida.net).

For light sheet imaging of mouse brains, intact whole mouse brains expressing TH were cleared using iDISCO and imaged by projected light sheet microscopy.

Cell detection

Cell nuclei from cre-positive cells in brains from the GENSATcreBrains project were marked using a machine learning classifier built by training a convolution neural network using NeuroInfo.

Atlas registration and cell mapping

Brain volumes compiled from sections and imaged by light sheet microscopy were aligned to the Allen Institute for Brain Science's Mouse Brain Atlas (mouse.brain-map.org) using NeuroInfo. Automated sequential section alignment was performed using proprietary image registration methods. This method accounts for histologic distortion and animal variation for 2D and 3D brain images (Hooks, et. al., 2018; Tappan, et. al., 2018), and maintains transforms for every section.

Following alignment, cell markers were mapped to the Allen Atlas reference space using the same geometric transforms found for each matched brain image volume. Counts were then partitioned by, and tallied within, the brain regions specified by the Allen Atlas.

Results: Aligning Serial Sections and Compiling a 3D Volume

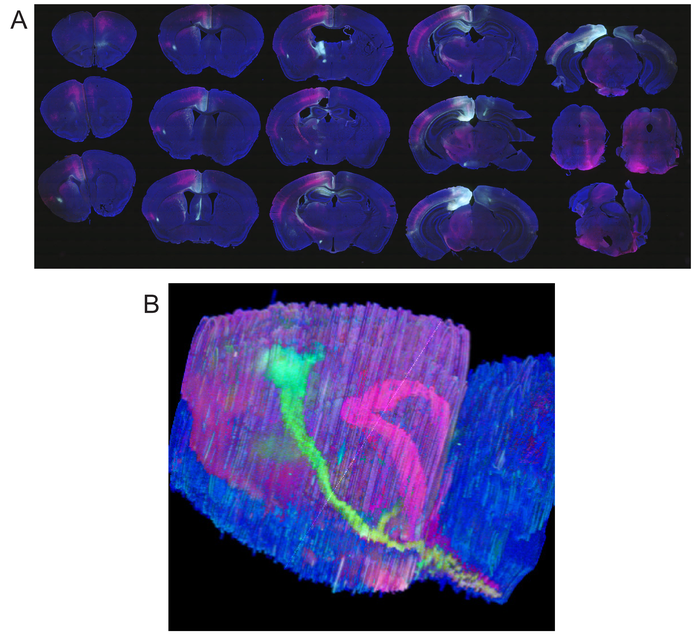

Figure 1. In BrainMaker, fluorescent serial sections (A) are automatically contoured based on contrast and edge strength. Image registration then finds a geometric transform that best maps points in extracted sections onto corresponding points in the next section. For each pair of adjacent sections, a transform is estimated that aligns sections by their centers, shape, and image content. Multiple hypotheses are formed to test whether a section has been flipped during float mount processing. Individual section transforms are composed to align all images to the same reference frame and compile a 3D volume. A maximum intensity projection (B) through the resulting 3D brain volume displays the path of a cortical injection.

Results: Registering Distorted Sections to an Atlas

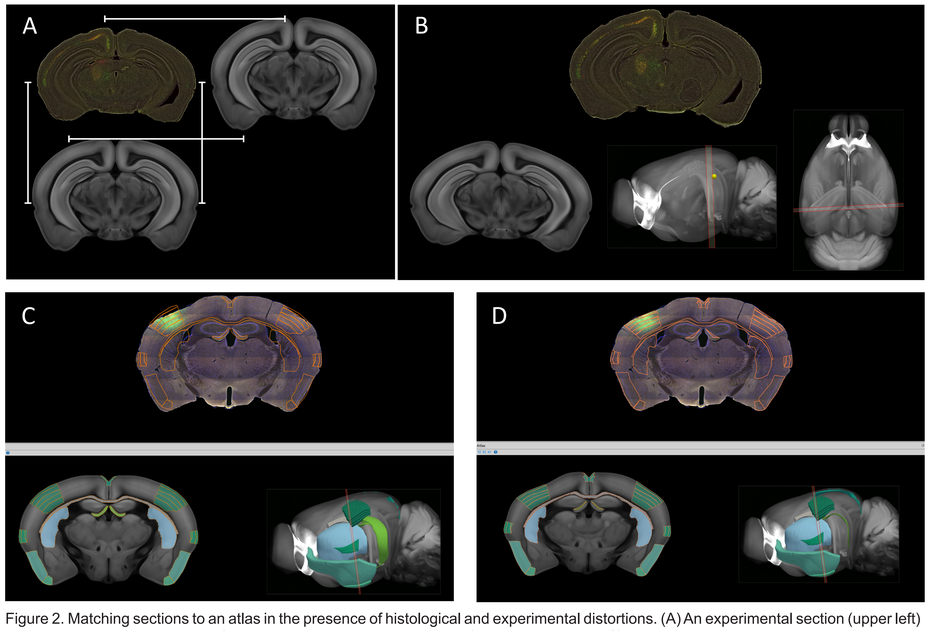

Figure 2. Matching sections to an atlas in the presence of histological and experimental distortions. (A) An experimental section (upper left) and the corresponding section from the Allen average mouse brain (upper right, lower left). Images have equivalent scaling, but tissue compression vertically and horizontally is observable. (B) The same experimental section matched to the Allen average mouse brain in the CCF coordinate system. The red line indicates the position of the matching section in the CCF. The transform estimated to match the experimental section to the atlas accounts for oblique angles, due to cutting angle and tissue embedding orientation, of the section in the CCF. (C) An experimental section (C, top) exhibiting cortical distortion due to an experimental model. A linear transform was utilized to find the matching section in the CCF (C, bottom), but could not sufficiently account for the experimental deformation. Orange regions indicate automatically delineated anatomies from the atlas. Corresponding regions are shaded in the average mouse brain (C, bottom). (D) The same section matched to the Allen average mouse brain using an estimated non-linear transform. Regions are indicated as in (C). Rat brain sections registered to the Waxholm Space are included as images in supplemental images included with this presentation.

Results: Registering Volumes to an Atlas

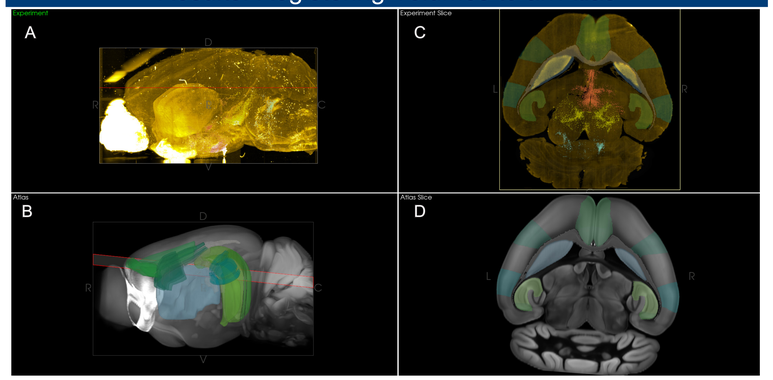

Figure 3. Registering image volumes of cleared mouse brain volumes to a standardized reference space.(A) A projection through a cleared experimental mouse brain. (B) The CCF brain with multiple brain regions indicated and the equivalent experimental section (red, angled box). (C) A transverse section from the experimental brain volume registered to the CCF with multiple brain regions highlighted. (D) The same transverse section position from C in the registered CCF brain.

Results: Cell Detection using Deep Machine Learning

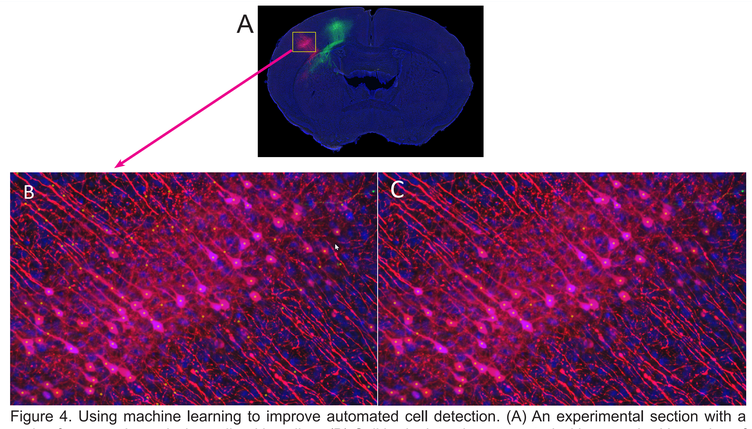

Figure 4. Using machine learning to improve automated cell detection. (A) An experimental section with a region for example analysis outlined in yellow. (B) Cell body detection attempted with a standard Lapacian of Gaussian detector. Detected cells are indicated by yellow dots. (C) The same region displaying cells detected by a convolutional neural network derived machine learning classifier.

Results: Mapping Measurements to a Reference Space

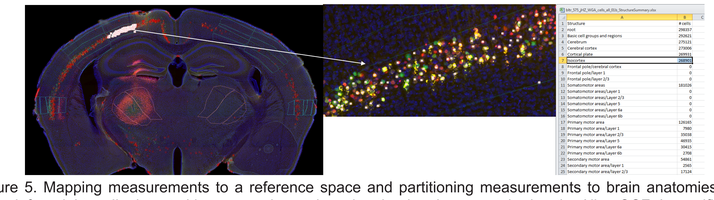

Figure 5. Mapping measurements to a reference space and partitioning measurements to brain anatomies. From left to right, cells detected in an experimental section that has been matched to the Allen CCF. A specific cortical region with yellow cells is displayed in the middle panel. Once a transform that matches the experimental section to the Allen average mouse brain, that transform is used to map cell coordinates to specific anatomical regions in the Allen Mouse Brain Atlas and detected cells can be tallied in each brain region (right most table).

Discussion

These example cases demonstrate our recent technological advances for registering images of mouse brain sections and analytical measurements to the Allen Mouse Brain Atlas coordinate system, even in the presence of histological and experimental distortions. Using advanced algorithms, we were able to accommodate for section-to-section and volumetric distortions, and then accurately map cell populations to the Allen Atlas. These cell populations can be distinguished as being in particular brain regions and compared across animals and experimental groups.

Acknowledgements and References

- The Mouse Brain Atlas was obtained from the Allen Institute for Brain Science.
- Hooks BM, et. al., "Topographic precision in sensory and motor corticostriatal projections varies across cell type and cortical area," Nature Communications, vol. 9, no. 1, p. 3549, Sep. 2018.
- Tappan SJ, et. al. 2018. Automatic navigation system for the mouse brain. bioRxiv doi.org: 10.1101/442558
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