

Modular strategies for spatial mapping of diverse cell type data of the mouse brain

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²³ **Abstract**

²⁴ Large-scale, international collaborative efforts by members of the BRAIN Initiative Cell
²⁵ Census Network (BICCN) consortium have recently begun aggregating the most compre-
²⁶ hensive reference database to date for diverse cell type profiling of the mouse brain, which
²⁷ encompasses over 40 different multi-modal profiling techniques from more than 30 research
²⁸ groups. One central challenge for this integrative effort across different investigators and
²⁹ laboratories has been the need to map these unique datasets into common reference spaces
³⁰ such that the spatial, structural, and functional information from different cell types can be
³¹ jointly analyzed across modalities. However, significant variations in the acquisition, tissue
³² processing, and imaging techniques across data types makes mapping such diverse data a
³³ multifarious problem. Different data types exhibit unique tissue distortion and signal char-
³⁴ acteristics that precludes a single mapping strategy from being generally applicable across
³⁵ all cell type data. Diverse, and often specialized, mapping approaches are needed to address
³⁶ the unique barriers present in each modality. This work highlights modular atlas mapping
³⁷ strategies developed across three separate BICCN studies using the Advanced Normalization
³⁸ Tools Ecosystem (ANTsX) to map spatial transcriptomic (MERFISH) and high-resolution
³⁹ morphology (fMOST) mouse brain data into the Allen Common Coordinate Framework
⁴⁰ (AllenCCFv3), and developmental (MRI and LSFM) data into the Developmental Common
⁴¹ Coordinate Framework (DevCCF). We discuss both common mapping strategies that can be
⁴² shared across modalities, and targeted strategies driven by specific challenges from each data
⁴³ type. Novel open-source contributions, made publicly available through ANTSX, include a
⁴⁴ generic velocity flow-based approach for continuously mapping developmental trajectories
⁴⁵ such as that characterizing the DevCCF as well as an automated framework for determining
⁴⁶ structural morphology made possible through the leveraging of public resources such as the
⁴⁷ AllenCCFv3 and the DevCCF. Finally, we provide general guidance to aid investigators in
⁴⁸ their efforts to tailor these strategies to address unique challenges in their data without the
⁴⁹ need to develop additional specialized software.

50 1 Introduction

51 Over the past decade there have been significant advancements in mesoscopic single-cell anal-
52 ysis of the mouse brain. It is now possible to track single neurons in mouse brains¹, observe
53 whole brain developmental changes on a cellular level², associate brain regions and tissues
54 with their genetic composition³, and locally characterize neural connectivity⁴. Much of these
55 scientific achievements have been made possible due to breakthroughs in high resolution cell
56 profiling and imaging techniques that permit submicron, multi-modal, 3D characterizations
57 of whole mouse brains. Among these include advanced techniques such as micro-optical
58 sectioning tomography⁶, tissue clearing^{1,7}, spatial transcriptomics⁹, and single-cell genomic
59 profiling¹⁰, which have greatly expanded the resolution and specificity of single-cell measure-
60 ments in the brain.

61 Recent efforts by the National Institutes of Health's Brain Research Through Advancing
62 Innovative Neurotechnologies (BRAIN) Initiative has pushed for large-scale, international
63 collaborative efforts to utilize these advanced single cell techniques to create a comprehensive
64 reference database for high-resolution transcriptomic, epigenomic, structural and imaging
65 data of the mouse brain. This consortium of laboratories and data centers, known as the
66 BRAIN Initiative Cell Census Network (BICCN), has to date archived datasets encompassing
67 over 40 different multi-modal profiling techniques from more than 30 research groups, each
68 providing unique characterizations of distinct cell types in the brain¹¹. Several of these
69 modalities have been further developed into reference atlases to facilitate spatial alignment
70 of individual brains and different data types into a common coordinate framework (CCF),
71 thus allowing diverse single-cell information to be integrated and analyzed in tandem. The
72 most notable of these atlases is the Allen Mouse Brain Common Coordinate Framework
73 (AllenCCFv3)¹², which serves as the primary target coordinate space to which the majority
74 of BICCN mouse data are mapped. Other atlases include modality-specific atlases¹³⁻¹⁵, and
75 spatiotemporal atlases^{16,17} for the developing mouse brain.

76 **1.1 Mouse brain mapping**

77 The cross-modality associations that can be learned from mapping different cell type data
78 into a CCF is critical for improving our understanding of the complex relationships between
79 cellular structure, morphology, and genetics in the brain. However, finding an accurate map-
80 ping between each individual mouse brain and a CCF is a challenging and heterogeneous task.
81 There is significant variance in the acquisition, fixation and imaging protocols across different
82 cell type data, and different tissue processing and imaging methods can potentially introduce
83 modality specific tissue distortion and signal differences^{18,19}. Certain modalities can have
84 poor intensity correspondence with the CCF, making image alignment less robust. Studies
85 targeting specific regions or cell types can lead to missing anatomical correspondences. Other
86 considerations include artifacts such as tissue distortion, holes, bubbles, folding, tears, and
87 missing sections in the data that often require manual correction^{20–23}. Given the diversity
88 of these challenges, it is unlikely any single mapping approach can be generally applicable
89 across all cell type data. Diverse, and often specialized, strategies are needed to address the
90 unique barriers present for mapping each modality.

91 Existing solutions to address mapping cell type data into the AllenCCFv3 falls broadly into
92 three main categories. The first consists of integrated processing platforms that directly
93 provide mapped data to the users. These include the Allen Brain Cell Atlas²⁴ for the Allen
94 Reference Atlas (ARA) and associated data, the Brain Architecture Portal²⁵ for combined
95 ex vivo radiology and histology data, OpenBrainMap²⁶ for connectivity data, and the Image
96 and Multi-Morphology Pipeline²⁷ for high resolution morphology data. These platforms
97 provide users online access to pre-processed, multi-modal cell type data that are already
98 mapped to the AllenCCFv3. The platforms are designed such that the data is interactively
99 manipulated by users through integrated visualization software that allow users to spatially
100 manipulate and explore each dataset within the mapped space. While highly convenient
101 for investigators who are interested in studying the specific modalities provided by these
102 platforms, these systems can be limited in flexibility and more general applicability. The
103 mapping software and pipelines are typically developed specifically with the data type and
104 platform in mind, and the software is limited public availability. Investigators will find it

¹⁰⁵ difficult to apply the same mapping to their own data without direct collaboration with the
¹⁰⁶ platform owners.

¹⁰⁷ The second category are specialized approaches specifically designed for mapping one or
¹⁰⁸ more modalities into a CCF. These approaches use combinations of specialized manual and
¹⁰⁹ automated processes that address specific challenges in each modality. Examples include ap-
¹¹⁰ proaches for mapping histology^{28–30}, magnetic resonance imaging (MRI)³⁷, micro-computed
¹¹¹ tomography (microCT)^{35,37}, light-sheet fluorescence microscopy (LSFM)^{34,36–39}, fluorescence
¹¹² micro-optical sectioning tomography (fMOST)^{15,40} and transcriptomic data^{41–43}. As special-
¹¹³ ized approaches, these techniques tend to boast higher mapping accuracy, robustness, and
¹¹⁴ ease of use when ran with applicable modalities. Conversely, their specialized designs often
¹¹⁵ rely on base assumptions regarding the data type that can make them rigid and difficult
¹¹⁶ to adapt for new modalities or unexpected artifacts and distortions in the data. Retooling
¹¹⁷ these specialize software to use with new data can require significant development, validation
¹¹⁸ time, and engineering expertise that may not be readily available for all investigators.

¹¹⁹ The last category are modular mapping approaches constructed using general image analy-
¹²⁰ sis toolkits, which are software packages that include varied collections of image processing,
¹²¹ segmentation and registration tools that have been previously developed, and validated for
¹²² multiple application areas. Examples of such toolkits include elastix⁴⁴, Slicer3D⁴⁵, ANTsX⁴⁶,
¹²³ and several others which have all been applied towards mouse brain spatial mapping. The
¹²⁴ main challenge, in these mouse-specific study scenarios, is that tailored pipelines often need
¹²⁵ be constructed from available software components. Investigators must therefore be familiar
¹²⁶ with the these tools for formulating new or adapting existing pipelines. However, in com-
¹²⁷ parison to previously described specialized mapping approaches, these approaches are often
¹²⁸ easier to create and prone to robustness, being typically constructed from pipelin compo-
¹²⁹ nents which have been previously vetted in other contexts. In this work, we highlight such
¹³⁰ mapping strategies designed using the ANTsX framework to map three distinct mouse cell
¹³¹ type data with different characteristics into existing CCFs.

¹³² **1.2 Advanced Normalization Tools (ANTsX)**

¹³³ The Advanced Normalization Tools Ecosystem (ANTsX) has been used in a number of
¹³⁴ applications for mapping mouse brain data as part of core processing steps in various
¹³⁵ workflows^{30,47–50}, particularly its pairwise, intensity-based image registration capabilities and
¹³⁶ bias field correction. Historically, ANTsX development is originally based on fundamental
¹³⁷ approaches to image mapping^{51–53}, particularly in the human brain, which has resulted
¹³⁸ in core contributions to the field such as the widely-used Symmetric Normalization (SyN)
¹³⁹ algorithm⁵⁴. Since its development, various independent platforms have been used to eval-
¹⁴⁰ uate ANTsX image registration capabilities in the context of different application foci which
¹⁴¹ include multi-site brain MRI data⁵⁵, pulmonary CT data⁵⁶, and most recently, multi-modal
¹⁴² brain registration in the presence of tumors⁵⁷.

¹⁴³ Apart from its registration capabilities, ANTsX comprises additional functionality such
¹⁴⁴ as template generation⁵⁸, intensity-based segmentation⁵⁹, preprocessing^{60,61}, deep learning
¹⁴⁵ networks⁴⁶, and other utilities relevant to brain mapping (see Table 1). The use of the toolkit
¹⁴⁶ has demonstrated high performance in multiple application areas (e.g., consensus labeling⁶²,
¹⁴⁷ brain tumor segmentation⁶³, and cardiac motion estimation⁶⁴). Importantly, ANTsX is built
¹⁴⁸ on the Insight Toolkit (ITK)⁶⁵ deriving benefit from the open-source community of sci-
¹⁴⁹ entists and programmers as well as providing an important resource for algorithmic develop-
¹⁵⁰ ment, evaluation, and improvement. In this paper we demonstrate how ANTsX provides a
¹⁵¹ comprehensive toolset provides the basis to develop modular frameworks for mapping di-
¹⁵² verse mouse cell type data into common coordinate frameworks (CCFs). Specifically, we
¹⁵³ highlight its application for mapping data from three separate BICCN projects focused on
¹⁵⁴ distinct data types: morphology data using fluorescence micro-optical sectioning tomog-
¹⁵⁵ raphy (fMOST), spatial transcriptomics from multiplexed error-robust fluorescence in situ
¹⁵⁶ hybridization (MERFISH) data, and time-series developmental data using light sheet fluores-
¹⁵⁷ cence microscopy (LSFM) and magnetic resonance imaging (MRI). We describe both shared
¹⁵⁸ and targeted strategies developed to address the specific challenges of these modalities.

¹⁵⁹ **1.3 Novel ANTsX-based open-source contributions**

¹⁶⁰ We introduce two novel inclusions to the ANTsX toolset that were developed as part of
¹⁶¹ the MRI mapping and analysis pipeline for the Developmental Common Coordinate Frame-
¹⁶² work (DevCCF). Consistent with previous ANTsX development, newly introduced capa-
¹⁶³ bilities introduced below are available through ANTsX (specifically, via R and Python
¹⁶⁴ ANTsX packages), and illustrated through self-contained examples in the ANTsX tuto-
¹⁶⁵ rial (<https://tinyurl.com/antsxtutorial>) with a dedicated GitHub repository specific to this
¹⁶⁶ work (<https://github.com/ntustison/ANTsXMouseBrainMapping>). To complement stan-
¹⁶⁷ dard preprocessing steps (e.g., bias correction, brain masking), additional mouse brain spe-
¹⁶⁸ cific tools have also been introduced to the ANTsX ecosystem, such as section reconstruction
¹⁶⁹ and landmark-based alignment with corresponding processing scripts (<https://github.com/>
¹⁷⁰ [dontminchenit/CCFAAlignmentToolkit](https://github.com/dontminchenit/CCFAAlignmentToolkit)).

¹⁷¹ **1.3.1 The DevCCF velocity flow model**

¹⁷² Recently, the Developmental Common Coordinate Framework (DevCCF) was introduced to
¹⁷³ the mouse brain research community as a public resource¹⁶ comprising symmetric atlases
¹⁷⁴ of multimodal image data and anatomical segmentations defined by developmental ontol-
¹⁷⁵ ogy. These templates sample the mouse embryonic days (E) 11.5, E13.5, E15.5, E18.5 and
¹⁷⁶ postnatal day (P) 4, P14, and P56. Modalities include light sheet fluorescence microscopy
¹⁷⁷ (LSFM) and at least four MRI contrasts per developmental stage. Anatomical parcellations
¹⁷⁸ are also available for each time point and were generated from ANTsX-based mappings of
¹⁷⁹ gene expression and other cell type data. Additionally, the P56 template was integrated
¹⁸⁰ with the AllenCCFv3 to further enhance the practical utility of the DevCCF. These pro-
¹⁸¹ cesses, specifically template generation and multi-modal image mapping, were performed
¹⁸² using ANTsX functionality in the presence of image mapping difficulties such as missing
¹⁸³ data and tissue distortion¹⁶.

¹⁸⁴ Given the temporal gaps in the discrete set of developmental atlases, we also provide an
¹⁸⁵ open-source framework for inferring correspondence within the temporally continuous do-

¹⁸⁶ main sampled by the existing set of embryonic and postnatal atlases of the DevCCF. This
¹⁸⁷ recently developed functionality permits the generation of a diffeomorphic velocity flow trans-
¹⁸⁸ formation model⁶⁶, influenced by previous work⁶⁷. The resulting time-parameterized veloc-
¹⁸⁹ ity field spans the stages of the DevCCF where mappings between any two continuous time
¹⁹⁰ points within the span bounded by the E11.5 and P56 atlases is determined by integration
¹⁹¹ of the optimized velocity field.

¹⁹² 1.3.2 Automated structural parcellations of the mouse brain

¹⁹³ In contrast to the pipeline development in human data⁴⁶, limited tools exist yet to cre-
¹⁹⁴ ate adequate training data for automated parcellations of the mouse brain. In addition,
¹⁹⁵ mouse brain data acquisition often has unique issues, such as lower data quality or sampling
¹⁹⁶ anisotropy which limits its applicability to high resolution resources (e.g., AllenCCFv3, De-
¹⁹⁷ vCCF), specifically with respect to the corresponding granular brain parcellations derived
¹⁹⁸ from numerous hours of expert annotation leveraging multimodal imaging resources.

¹⁹⁹ Herein, we introduce a mouse brain parcellation pipeline for T2-weighted (T2-w) MRI com-
²⁰⁰ prising two novel deep learning components: two-shot learning brain extraction from data
²⁰¹ augmentation of two ANTsX templates generated from two open datasets^{68,69} and single-
²⁰² shot brain parcellation derived from the AllenCCFv3 labelings mapped to the corresponding
²⁰³ DevCCF P56 T2-w component. Although we anticipate that this pipeline will be benefi-
²⁰⁴ cial to the research community, this work demonstrates more generally how one can leverage
²⁰⁵ ANTsX tools for developing quantitative mouse brain morphological tools using only publicly
²⁰⁶ available resources. Evaluation is performed on an independent open dataset⁷⁰ comprising
²⁰⁷ longitudinal acquisitions of multiple specimens.

208 **2 Results**

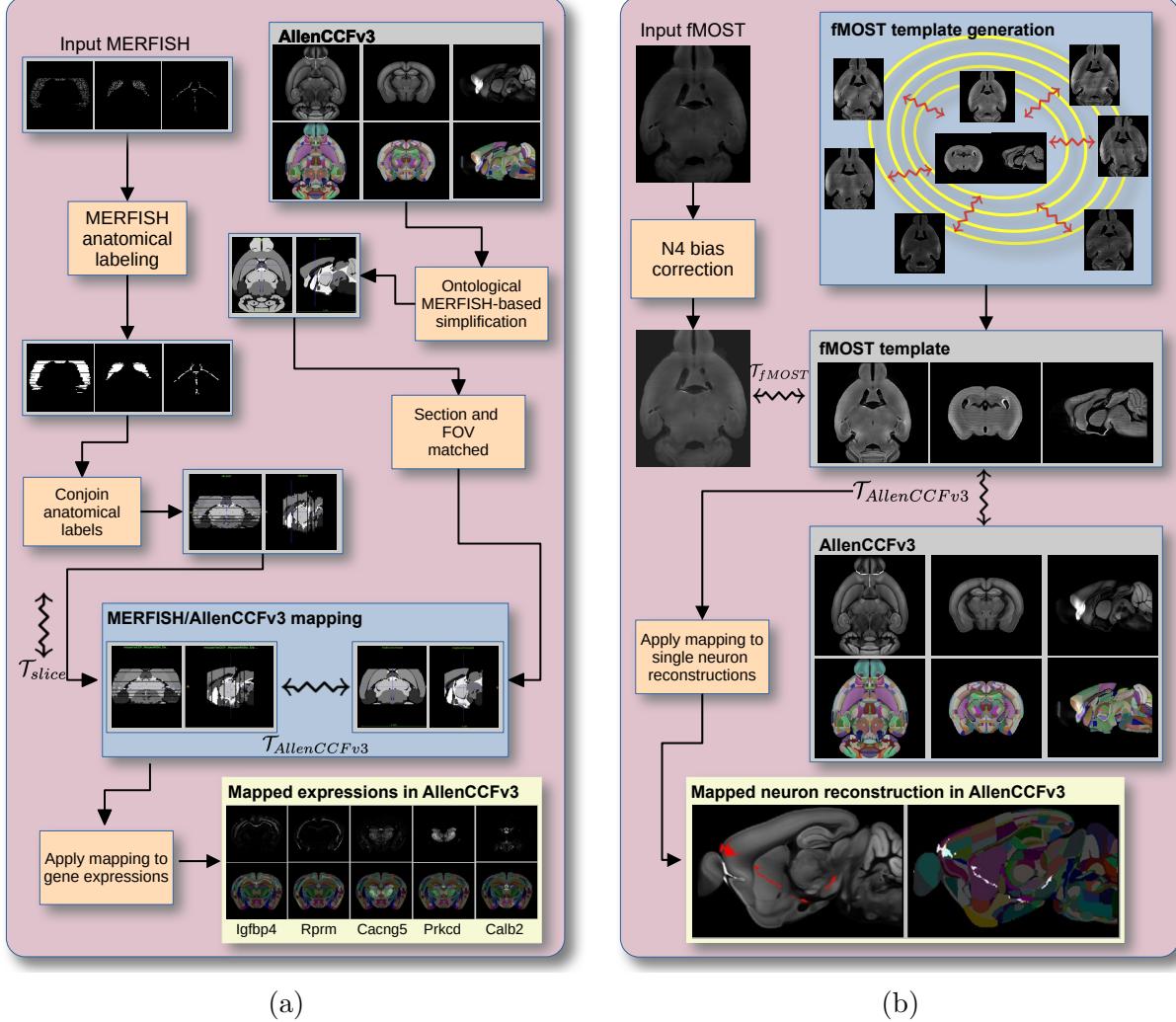


Figure 1: Diagram of the two ANTsX-based pipelines for mapping (a) MERFISH and (b)fMOST data into the space of AllenCCFv3. Each generates the requisite transforms, \mathcal{T} , to map individual images.

209 **2.1 AllenCCFv3 brain image mapping**

210 **2.1.1 Mapping multiplexed error-robust fluorescence *in situ* hybridization
211 (MERFISH) data**

212 **Overview.** The ANTsX framework was used to develop a pipeline for mapping multiplexed
213 error-robust fluorescence *in situ* hybridization (MERFISH) spatial transcriptomic mouse

214 data onto the AllenCCFv3 (see Figure 1(a)). This pipeline, used recently in creating a
215 high-resolution transcriptomic atlas of the mouse brain⁵⁰, performs mappings by first gen-
216 erating anatomical labels from tissue related gene expressions in the MERFISH data, and
217 then spatially matching these labels to corresponding anatomical tissue parcellations in the
218 AllenCCFv3. The pipeline consists of MERFISH data specific preprocessing which includes
219 section reconstruction, mapping corresponding anatomical labels between AllenCCFv3 and
220 the spatial transcriptomic maps of the MERFISH data, and matching MERFISH sections to
221 the atlas space. Following pre-processing, two main alignment steps were performed: 1) 3-D
222 global affine mapping and section matching of the AllenCCFv3 into the MERFISH data and
223 2) 2D global and deformable mapping between each MERFISH section and matched AllenC-
224 CFv3 section. Mappings learned via each step in the pipeline are preserved and concatenated
225 to provide point-to-point correspondence between the original MERFISH data and AllenC-
226 CFv3, thus allowing individual gene expressions to be transferred into the AllenCCFv3.

227 **Data.** MERFISH mouse brain data was acquired using a previously detailed procedure⁵⁰.
228 Briefly, a brain of C57BL/6 mouse was dissected according to standard procedures and placed
229 into an optimal cutting temperature (OCT) compound (Sakura FineTek 4583) in which it
230 was stored at -80°C. The fresh frozen brain was sectioned at 10 μ m on Leica 3050 S cryostats
231 at intervals of 200 μ m to evenly cover the brain. A set of 500 genes were imaged that had
232 been carefully chosen to distinguish the ~ 5200 clusters of our existing RNAseq taxonomy.
233 For staining the tissue with MERFISH probes, a modified version of instructions provided by
234 the manufacturer was used⁵⁰. Raw MERSCOPE data were decoded using Vizgen software
235 (v231). Cell segmentation was performed⁷¹. In brief, cells were segmented based on DAPI
236 and PolyT staining using Cellpose⁷². Segmentation was performed on a median z-plane
237 (fourth out of seven) and cell borders were propagated to z-planes above and below. To
238 assign cluster identity to each cell in the MERFISH dataset, we mapped the MERFISH cells
239 to the scRNA-seq reference taxonomy.

240 **Evaluation.** Alignment of the MERFISH data into the AllenCCFv3 was qualitatively as-
241 sessed by an expert anatomist at each iteration of the registration using known correspon-
242 dence of gene markers and their associations with the AllenCCFv3. As previously reported⁵⁰,

²⁴³ further assessment of the alignment showed that, of the 554 terminal regions (gray matter
²⁴⁴ only) in the AllenCCFv3, only seven small subregions were missed from the MERFISH
²⁴⁵ dataset: frontal pole, layer 1 (FRP1), FRP2/3, FRP5; accessory olfactory bulb, glomerular
²⁴⁶ layer (AOBgl); accessory olfactory bulb, granular layer (AOBgr); accessory olfactory bulb,
²⁴⁷ mitral layer (AOBmi); and accessory supraoptic group (ASO).

²⁴⁸ 2.1.2 Mapping fluorescence micro-optical sectioning tomography (fMOST) data

²⁴⁹ **Overview.** We developed a pipeline for mapping fluorescence micro-optical sectioning to-
²⁵⁰ mography (fMOST) mouse brain images into the AllenCCFv3 (see Figure 1(b)). The pipeline
²⁵¹ is adapted from previously developed frameworks for human brain mapping⁵⁸, and uses a
²⁵² modality specific (fMOST) average atlas to assist in the image registration and mapping.
²⁵³ This approach has been well validated in human studies^{73–75}, and successfully used in other
²⁵⁴ mouse data^{12,15,34}. Briefly, we construct an intensity- and shape-based average fMOST atlas
²⁵⁵ using 30 fMOST images to serve as an intermediate registration target for mapping fMOST
²⁵⁶ images from individual specimens into the AllenCCFv3. Preprocessing steps include down-
²⁵⁷ sampling to match the 25 μ m isotropic AllenCCFv3, acquisition-based stripe artifact removal,
²⁵⁸ and inhomogeneity correction⁶¹. Preprocessing also includes a single annotation-driven reg-
²⁵⁹ istration to establish a canonical mapping between the fMOST atlas and the AllenCCFv3.
²⁶⁰ This step allows us to align expert determined landmarks to accurately map structures
²⁶¹ with large morphological differences between the modalities, which are difficult to address
²⁶² using standard approaches. Once this canonical mapping is established, standard intensity-
²⁶³ based registration is used to align each new fMOST image to the fMOST specific atlas.
²⁶⁴ This mapping is concatenated with the canonical fMOST atlas-to-AllenCCFv3 mapping to
²⁶⁵ further map each individual brain into the latter without the need to generate additional
²⁶⁶ landmarks. Transformations learned through this mapping can be applied to single neuron
²⁶⁷ reconstructions from the fMOST images to evaluate neuronal distributions across different
²⁶⁸ specimens into the AllenCCFv3 for the purpose of cell census analyses.

²⁶⁹ **Data.** The high-throughput and high-resolution fluorescence micro-optical sectioning to-
²⁷⁰ mography (fMOST)^{76,77} platform was used to image 55 mouse brains containing gene-defined

271 neuron populations, with sparse transgenic expression^{78,79}. In short, the fMOST imaging
272 platform results in 3-D images with voxel sizes of $0.35 \times 0.35 \times 1.0 \mu\text{m}^3$ and is a two-channel
273 imaging system where the green channel displays the green fluorescent protein (GFP) labeled
274 neuron morphology and the red channel is used to visualize the counterstained propidium
275 iodide cytoarchitecture. The spatial normalizations described in this work were performed
276 using the red channel, which offered higher tissue contrast for alignment, although other
277 approaches are possible including multi-channel registration.

278 **Evaluation.** Evaluation of the canonical fMOST atlas to Allen CCFv3 mapping was per-
279 formed via quantitative comparison at each step of the registration and qualitative assess-
280 ment of structural correspondence after alignment by an expert anatomist. Dice values were
281 generated for the following structures: whole brain, 0.99; fimbria, 0.91; habenular com-
282 missure, 0.63; posterior choroid plexus, 0.93; anterior choroid plexus, 0.96; optic chiasm,
283 0.77; caudate putamen, 0.97. Similar qualitative assessment was performed for each fMOST
284 specimen including the corresponding neuron reconstruction data.

285 **2.2 The DevCCF velocity flow model**

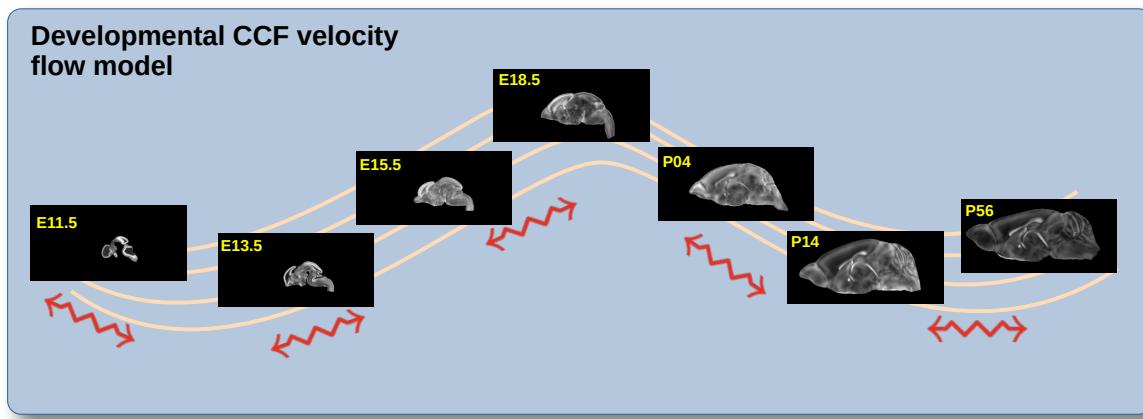


Figure 2: The spatial transformation between any two time points within the DevCCF longitudinal developmental trajectory is available through the use of ANTsX functionality for generating a velocity flow model.

286 To continuously interpolate transformations between the different stages of the DevCCF

atlases, a velocity flow model was constructed using DevCCF derived data and functionality recently introduced into both the ANTsR and ANTsPy packages. Both platforms include a complete suite of functions for determining dense correspondence from sparse landmarks based on a variety of transformation models ranging from standard linear models (i.e., rigid, affine) to deformable diffeomorphic models (e.g, symmetric normalization⁵⁴). The latter set includes transformation models for both the pairwise scenario and for multiple sets, as in the case of the DevCCF. ANTsX, being built on top of ITK, uses an ITK image data structure for the 4-D velocity field where each voxel contains the x , y , z components of the field at that point.

2.2.1 Data

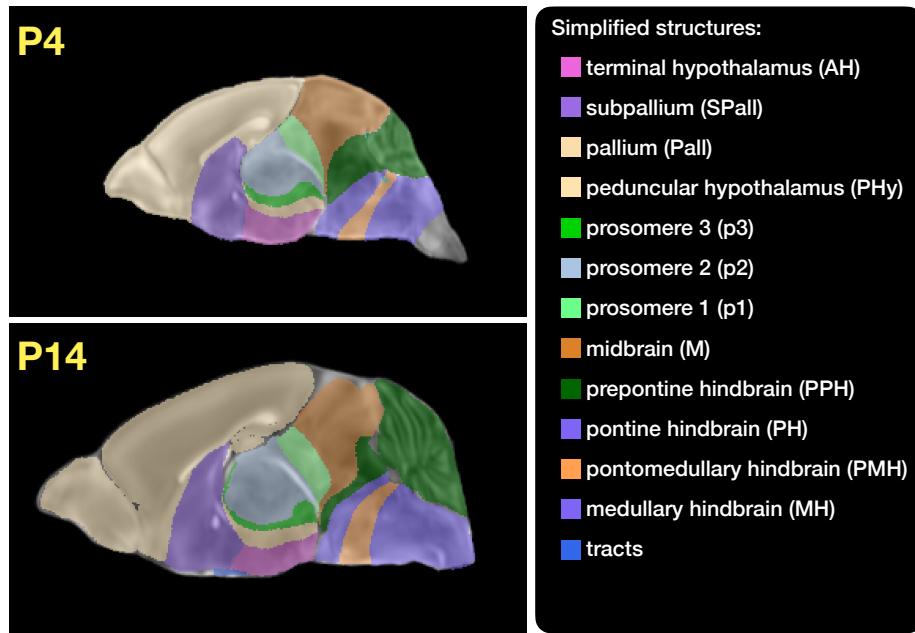


Figure 3: Annotated regions representing common labels across developmental stages which are illustrated for both P4 and P14.

Labeled annotations are available as part of the original DevCCF and reside in the space of each developmental template which range in resolution from $31.5 - 50\mu\text{m}$. Across all atlases, the total number of labeled regions exceeds 2500. From these labels, a common set of 26 labels (13 per hemisphere) across all atlases were used for optimization and evaluation. These simplified regions include: terminal hypothalamus, subpallium, pallium, peduncular

302 hypothalamus, prosomere, prosomere, prosomere, midbrain, prepontine hindbrain, pontine
303 hindbrain, pontomedullary hindbrain, medullary hindbrain, and tracts (see Figure 3).

304 Prior to velocity field optimization, all data were rigidly transformed to DevCCF P56 using
305 the centroids of the common label sets. In order to determine the landmark correspondence
306 across DevCCF stages, the multi-metric capabilities of `ants.registration(...)` were used.
307 Instead of performing intensity-based pairwise registration directly on these multi-label im-
308 ages, each label was used to construct a separate fixed and moving image pair resulting in a
309 multi-metric registration optimization scenario involving 24 binary image pairs (each label
310 weighted equally) for optimizing diffeomorphic correspondence between neighboring time
311 point atlases using the mean squares metric and the symmetric normalization transform⁵⁴.

312 To generate the set of common point sets across all seven developmental atlases, the label
313 boundaries and whole regions were sampled in the P56 atlas and then propagated to each
314 atlas using the transformations derived from the pairwise registrations. We selected a sam-
315 pling rate of 10% for the contour points and 1% for the regional points for a total number
316 of points being per atlas being 173303 ($N_{contour} = 98151$ and $N_{region} = 75152$). Regional
317 boundary points were weighted twice as those of non-boundary points during optimization.

318 2.2.2 Optimization

319 The velocity field was optimized using the input composed of the seven corresponding point
320 sets and their associated weight values, the selected number of integration points for the
321 velocity field ($N = 11$), and the parameters defining the geometry of the spatial dimensions
322 of the velocity field. Thus, the optimized velocity field described here is of size [256, 182, 360]
323 (50 μm isotropic) \times 11 integration points for a total compressed size of a little over 2 GB.
324 This choice represented weighing the trade-off between tractability, portability, and accuracy.
325 However, all data and code to reproduce the results described (with possible variation in the
326 input parameters) are available in the dedicated GitHub repository.

327 The normalized time point scalar value for each atlas/point-set in the temporal domains [0, 1]
328 was also defined. Given the increasingly larger gaps in the postnatal timepoint sampling, we
329 made two adjustments. Based on known mouse brain development, we used 28 days for the

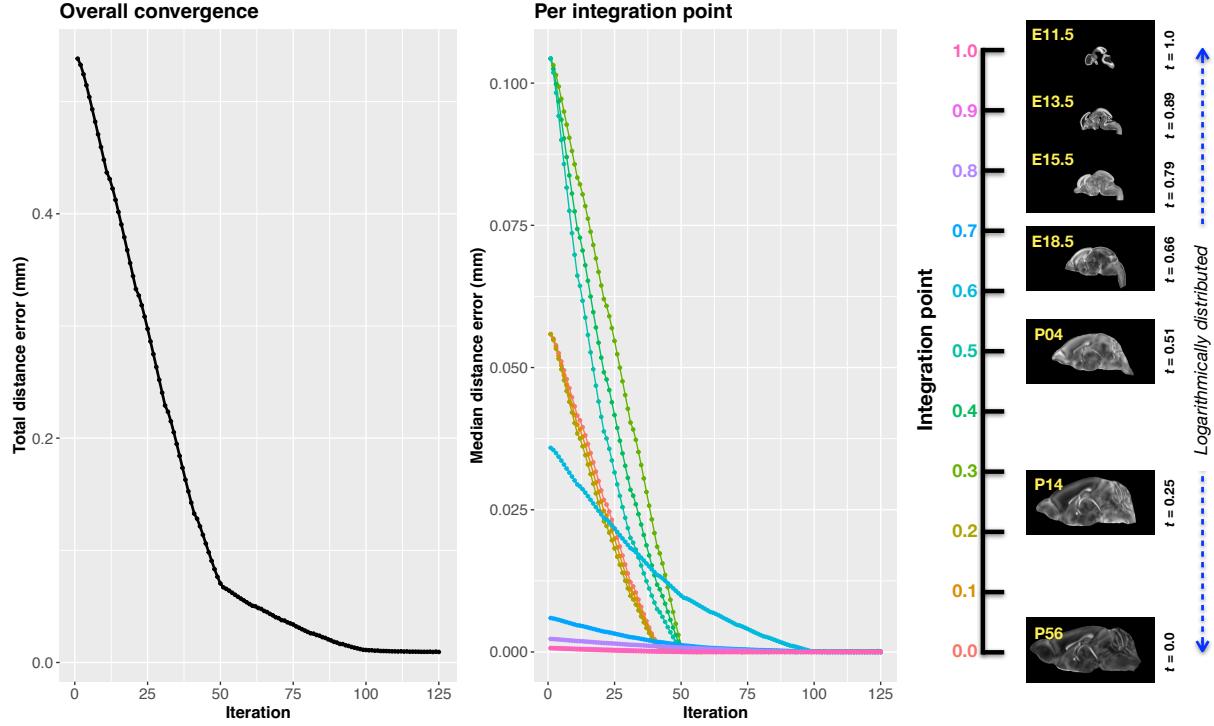


Figure 4: Convergence of the optimization of the velocity field for describing the transformation through the developmental stages from E11.5 through P56. Integration points in diagram on the right are color-coordinated with the center plot and placed in relation to the logarithmically situated temporal placement of the individual DevCCF atlases.

330 P56 data. We then computed the log transform of the adjusted set of time points prior to
 331 normalization between 0 and 1 (see the right side of Figure 4). This log transform, as part
 332 of the temporal normalization, significantly improved data spacing.

333 The maximum number of iterations was set to 200 with each iteration taking approximately
 334 six minutes on a 2020 iMac (processor, 3.6 GHz 10-Core Intel Core i9; memory, 64 GB 2667
 335 MHz DDR4) At each iteration we looped over the 11 integration points. At each integration
 336 point, the velocity field estimate was updated by warping the two immediately adjacent
 337 point sets to the integration time point and determining the regularized displacement field
 338 between the two warped point sets. As with any gradient-based descent algorithm, this field
 339 was multiplied by a small step size ($\delta = 0.2$) before adding to the current velocity field.
 340 Convergence is determined by the average displacement error over each of the integration
 341 points. As can be seen in the left panel of Figure 4, convergence occurred around 125
 342 iterations when the average displacement error over all integration points is minimized. The

343 median displacement error at each of the integration points also trends towards zero but at
 344 different rates.

345 **2.2.3 The transformation model**

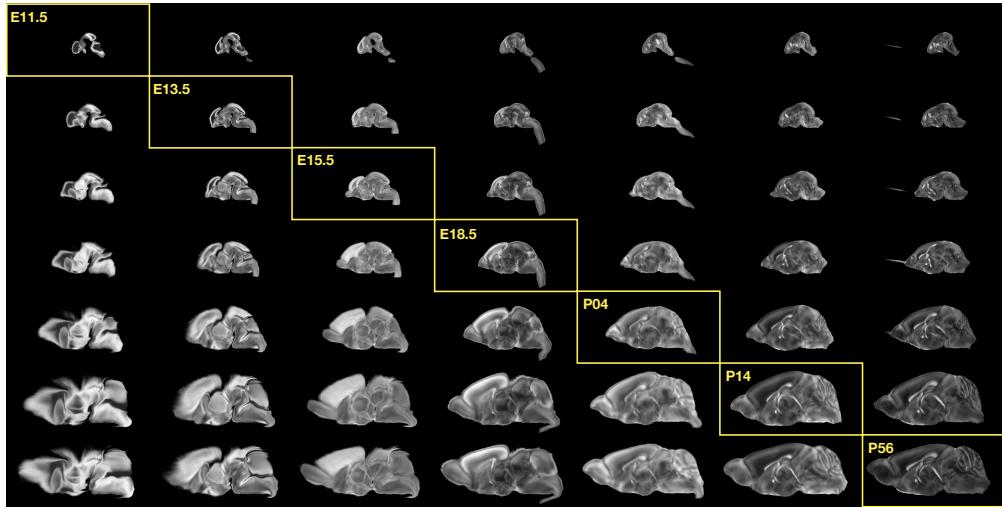


Figure 5: Mid-sagittal visualization of the effects of the transformation model in warping every developmental stage to the time point of every other developmental stage. The original images are located along the diagonal. Columns correspond to the warped original image whereas the rows represent the reference space to which each image is warped.

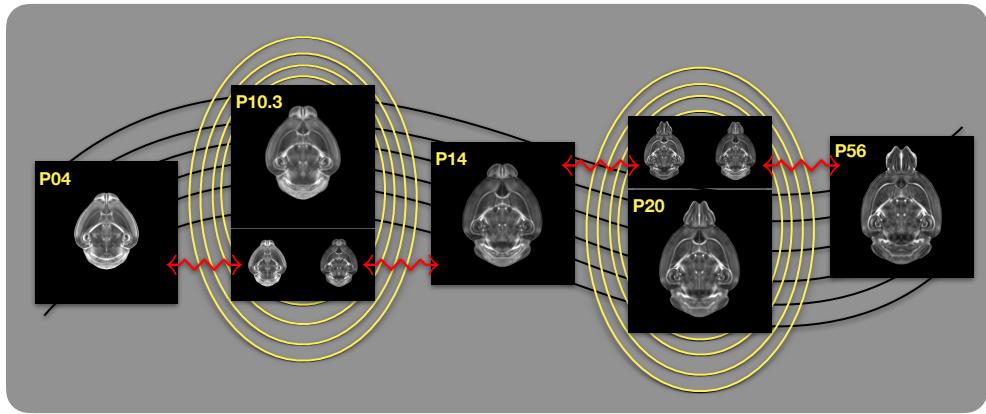


Figure 6: Illustration of the use of the velocity flow model for creating virtual templates at continuous time points not represented in one of the existing DevCCF time points. For example, FA templates at time point P10.3 and P20 can be generated by warping the existing temporally adjacent developmental templates to the target time point and using those images in the ANTsX template building process.

346 Once optimized, the resulting velocity field can be used to generate the deformable transform

347 between any two continuous points within the time interval bounded by E11.5 and P56. In
 348 Figure 5, we transform each atlas to the space of every other atlas using the DevCCF
 349 transform model. Additionally, one can use this transformation model to construct virtual
 350 templates in the temporal gaps of the DevCCF. Given an arbitrarily chosen time point
 351 within the normalized time point interval, the existing adjacent DevCCF atlases on either
 352 chronological side can be warped to the desired time point. A subsequent call to one of
 353 the ANTsX template building functions then permits the construction of the template at
 354 that time point. In Figure 6, we illustrate the use of the DevCCF velocity flow model for
 355 generating two such virtual templates for two arbitrary time points. Note that both of these
 356 usage examples can be found in the GitHub repository previously given.

357 2.3 The Mouse Brain Parcellation Pipeline

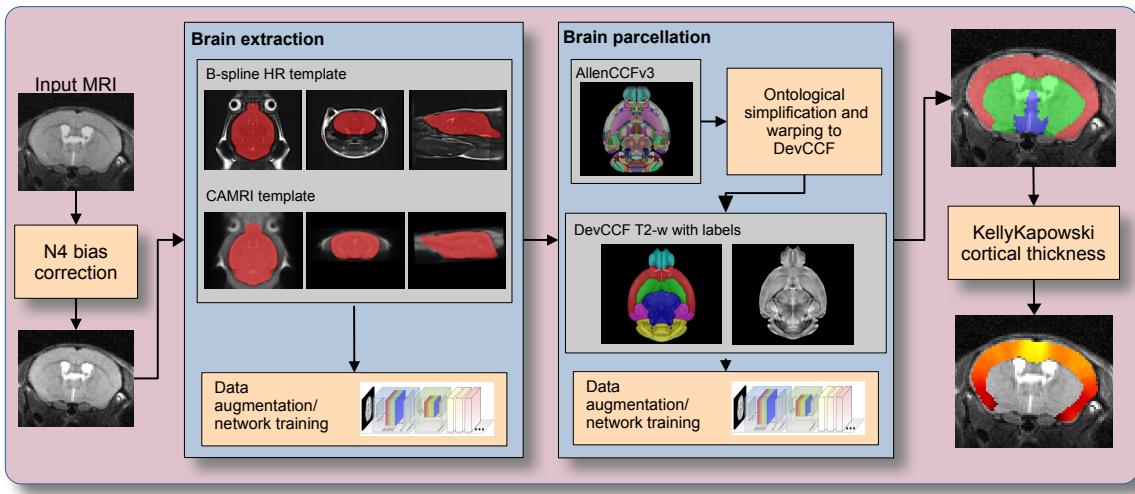


Figure 7: The mouse brain cortical parcellation pipeline integrating two deep learning components for brain extraction and brain parcellation prior to estimating cortical labels. Both deep learning networks rely heavily on data augmentation on templates built from open data and provide an outline for further refinement and creating alternative parcellations for tailored research objectives.

358 One of the most well-utilized pipelines in the ANTsX toolkit is the generation of corti-
 359 cal thickness maps in the human brain from T1-weighted MRI. Starting with the novel
 360 Diffeomorphic Registration-based Cortical Thickness (DiReCT) algorithm⁸⁰, a complete al-
 361 gorithmic workflow was developed for both cross-sectional⁸¹ and longitudinal⁸² T1-weighted

³⁶² MR image data. This contribution was later refactored using deep learning⁴⁶ leveraging the
³⁶³ earlier results⁸¹ for training data.

³⁶⁴ In the case of the mouse brain, the lack of training data and/or tools to generate training
³⁶⁵ data making analogous algorithmic development difficult. In addition, mouse data is often
³⁶⁶ characterized by unique issues such as frequent anisotropic sampling which are often in sharp
³⁶⁷ contrast to the high resolution resources available within the community, e.g., AllenCCFv3
³⁶⁸ and DevCCF. Using ANTsX and other publicly available data resources, we developed a
³⁶⁹ complete mouse brain structural morphology pipeline as illustrated in Figure 7 and detailed
³⁷⁰ below.

³⁷¹ 2.3.1 Two-shot mouse brain extraction network

³⁷² In order to create a generalized mouse brain extraction network, we built whole-head tem-
³⁷³ plates from two publicly available datasets. The Center for Animal MRI (CAMRI) dataset⁶⁸
³⁷⁴ from the University of North Carolina at Chapel Hill consists of 16 T2-weighted MRI volumes
³⁷⁵ of voxel resolution $0.16 \times 0.16 \times 0.16 mm^3$. The second high-resolution dataset⁶⁹ comprises
³⁷⁶ 88 specimens each with three spatially aligned canonical views with in-plane resolution of
³⁷⁷ $0.08 \times 0.08 mm^2$ with a slice thickness of $0.5 mm$. These three orthogonal views were used to
³⁷⁸ reconstruct a single high-resolution volume per subject using a B-spline fitting algorithm de-
³⁷⁹ veloped in ANTsX⁸³. From these two datasets, two symmetric isotropic ANTsX templates⁵⁸
³⁸⁰ were generated analogous to the publicly available ANTsX human brain templates used in
³⁸¹ previous research⁸¹. Bias field simulation, intensity histogram warping, noise simulation,
³⁸² random translation and warping, and random anisotropic resampling in the three canoni-
³⁸³ cal directions were used for data augmentation in training a T2-weighted brain extraction
³⁸⁴ network.

³⁸⁵ 2.3.2 Single-shot mouse brain parcellation network

³⁸⁶ To create the network for generating a brain parcellation consistent with cortical thickness
³⁸⁷ estimation, we used the AllenCCFv3 and the associated `allensdk` Python library. Using

388 allensdk, a gross parcellation labeling was generated from the fine Allen CCFv3 labeling
 389 which includes the cerebral cortex, cerebral nuclei, brain stem, cerebellum, main olfactory
 390 bulb, and hippocampal formation. This labeling was mapped to the P56 component of
 391 the DevCCF. Both the T2-w P56 DevCCF and labelings, in conjunction with the data
 392 augmentation described previously for brain extraction, was used to train a brain parcellation
 393 network.

394 2.3.3 Evaluation

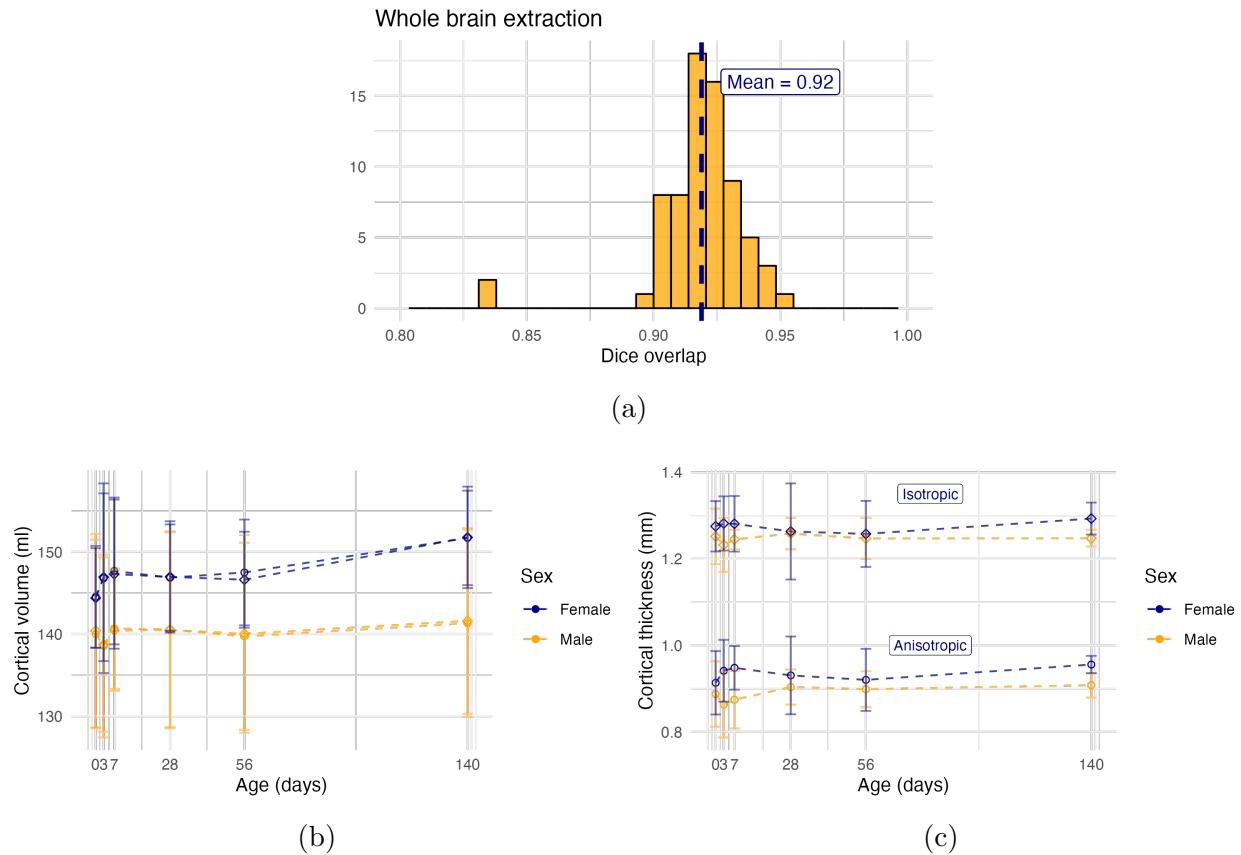


Figure 8: Evaluation of the ANTsX mouse brain extraction, parcellation, and cortical thickness pipeline on an independent dataset consisting of 12 specimens \times 7 time points = 84 total images. (a) Dice overlap comparisons with the provided brain masks provide generally good agreement with the brain extraction network. (b) Cortical volume measurements show similar average quantities over growth and development between the original anisotropic data and interpolated isotropic data. (c) These results contrast with the cortical thickness measurements which show that cortical thickness estimation in anisotropic space severely underestimates the actual values.

395 For evaluation, we used an additional publicly available dataset⁷⁰ which is completely in-
396 dependent from the data used in training the brain extraction and parcellation networks.
397 Data includes 12 specimens each imaged at seven time points (Day 0, Day 3, Week 1, Week
398 4, Week 8, Week 20) with available brain masks. In-plane resolution is $0.1 \times 0.1 mm^2$ with
399 a slice thickness of $0.5 mm$. Since the training data is isotropic and data augmentation in-
400 cludes downsampling in the canonical directions, each of the two networks learns mouse
401 brain-specific interpolation such that one can perform prediction on thick-sliced images, as,
402 for example, in these evaluation data, and return isotropic probability and thickness maps (a
403 choice available to the user). Figure 8 summarizes the results of the evaluation and compar-
404 ison between isotropic and anisotropic cortical measurements in male and female specimens.

405 **3 Discussion**

406 The diverse mouse brain cell type profiles gathered through BICCN and associated efforts
407 provides a rich multi-modal resource to the community. However, despite significant progress,
408 full integration of these valuable resources is not yet complete. Central to the data integra-
409 tion is a continued need to accurately map each unique dataset into common coordinate
410 frameworks (CCFs) so that they can be accessed in connection with each other. Addition-
411 ally, the ability to map novel cell type data in the future to these existing BICCN resources
412 is vital for effective utilization of this endeavor and the continuation of its goals. To meet
413 these needs, tools for mapping mouse cell type data must be both generally accessible to
414 a wide audience of investigators, and still capable of handling distinct challenges unique to
415 each data type.

416 In this work, we describe modular ANTsX-based pipelines developed to address the needs
417 of three BICCN projects that cover distinct cell type data, including spatial transcriptomic,
418 morphology, and developmental data. We highlight how a modular toolbox like ANTsX can
419 be tailored to address problems unique to each modality while still leveraging a variety of
420 ready-to-use powerful tools that have been externally validated.

421 Our MERFISH pipeline provides an example of how to map high-resolution spatial tran-
422 scriptomic data into the AllenCCFv3. Since full brain large-scale transcriptomics is still
423 rare and difficult to collect, the pipeline focuses on achieving the best possible anatomical
424 alignment and fully utilizing the available data. While the techniques employed for mapping
425 the sectioned data can be generally applicable to map other serial histology images, many
426 parts of the pipeline were designed to address very specific known alignment challenges in
427 the MERFISH data using a series of iterative registration steps. The pipeline shows how
428 general tools available in ANTsX can be adapted to target highly specialized problems in
429 mouse cell type data.

430 In contrast to the MERFISH pipeline, our fMOST pipeline was designed to be a more
431 general solution that can be employed in other modalities. The pipeline primarily uses
432 previously developed ANTsX preprocessing and atlasing tools to map fMOST data into the

433 AllenCCFv3. The key component of the pipeline is the use of a fMOST specific average
434 atlas to greatly simplify the image registration problem. This average atlas, also constructed
435 using pre-existing ANTsX tools, allows for a one-time canonical alignment from the fMOST
436 atlas to the AllenCCFv3 to be transferred and used for mapping new fMOST images. Lastly,
437 ANTsX provides point set transformation tools to allow the mappings found through the
438 pipeline to be directly applied to associated single-cell reconstructions from the fMOST data
439 to study neuronal morphology.

440 Our DevCCF pipeline shows the application of the toolkit for temporospatial developmental
441 data. ANTsX was crucial in providing necessary functionality for yielding high quality
442 output. For the generation of the individual developmental stage multi-modal, symmetric
443 templates, ANTsX is unique amongst image analysis software packages in providing existing
444 solutions for template generation which have been thoroughly vetted, including being used
445 in several studies over the years, and which continue to be under active refinement. At its
446 core, computationally efficient and quality template generation requires the use of precision
447 pairwise image mapping functionality which, historically, is at the origins of the ANTsX
448 ecosystem. Moreover, these mapping capabilities extend beyond template generation to the
449 mapping of other image data (e.g., gene expression maps) to a selected template for providing
450 further insight into the mouse brain.

451 With respect to the DevCCF, despite the significant expansion of available developmental age
452 templates beyond what existed previously, there are still temporal gaps in the DevCCF which
453 can be potentially sampled by future research efforts. However, pioneering work involving
454 time-varying diffeomorphic transformations allow us to continuously situate the existing
455 templates within a velocity flow model. This allows one to determine the diffeomorphic
456 transformation from any one temporal location to any other temporal location within the
457 time span defined by the temporal limits of the DevCCF. This functionality is built on
458 multiple ITK components including the B-spline scattered data approximation technique for
459 field regularization and velocity field integration. This velocity field model permits intra-
460 template comparison and the construction of virtual templates where a template can be
461 estimated at any continuous time point within the temporal domain. This novel application

462 can potentially enhance our understanding of intermediate developmental stages.

463 We also presented a mouse brain pipeline for brain extraction, parcellation, and cortical
464 thickness using single-shot and two-shot learning with data augmentation. This approach
465 attempts to circumvent (or at least minimize) the typical requirement of large training
466 datasets as with the human ANTsX pipeline analog. However, even given our initial success
467 on independent data, we fully anticipate that refinements will be necessary. Given that the
468 ANTsX toolkit is a dynamic effort undergoing continual improvement, we manually correct
469 cases that fail and use them for future training and refinement of network weights as we have
470 done for our human-based networks. Generally, these approaches provide a way to bootstrap
471 training data for manual refinement and future generation of more accurate deep learning
472 networks in the absence of other applicable tools.

473 The ANTsX ecosystem is a powerful framework that has demonstrated applicability to di-
474 verse cell type data in the mouse brain. This is further evidenced by the many software
475 packages that use various ANTsX components in their own mouse-specific workflows. In
476 and of itself, the extensive functionality of ANTsX makes it possible to create complete pro-
477 cessing pipelines without requiring the integration of multiple packages or lengthy software
478 development. These open-source components not only perform well but are available across
479 multiple platforms which facilitates the construction of tailored pipelines for individual study
480 solutions. These components are also supported by years of development not only by the
481 ANTsX development team but by the larger ITK community.

482 **4 Methods**

483 The following methods are all available as part of the ANTsX ecosystem with analogous
484 elements existing in both ANTsR (ANTs in R) and ANTsPy (ANTs in Python) with an
485 ANTs/ITK C++ core. However, most of the development for the work described below was
486 performed using ANTsPy. For equivalent calls in ANTsR, please see the ANTsX tutorial at
487 <https://tinyurl.com/antsxtutorial>.

488 **4.1 General ANTsX utilities**

489 Although they focus on distinct data types, the three pipelines presented share common
490 components that are generally applicable when mapping mouse cell type data. These include,
491 addressing intensity biases and noise in the data, image registration to solve the mapping,
492 creating custom templates and atlases from the data, and visualization of the results. Table
493 1 provides a brief summary of key general functionalities in ANTsX for addressing these
494 challenges.

495 **4.1.1 Preprocessing: bias field correction and denoising**

496 Bias field correction and image denoising are standard preprocessing steps in improving over-
497 all image quality in mouse brain images. The bias field, a gradual spatial intensity variation
498 in images, can arise from various sources such as magnetic field inhomogeneity or acquisition
499 artifacts, leading to distortions that can compromise the quality of brain images. Correct-
500 ing for bias fields ensures a more uniform and consistent representation of brain structures,
501 enabling more accurate quantitative analysis. Additionally, brain images are often suscep-
502 tible to various forms of noise, which can obscure subtle features and affect the precision
503 of measurements. Denoising techniques help mitigate the impact of noise, enhancing the
504 signal-to-noise ratio and improving the overall image quality. The well-known N4 bias field
505 correction algorithm⁶¹ has its origins in the ANTs toolkit which was implemented and intro-
506 duced into the ITK toolkit, i.e. `ants.n4_bias_field_correction(...)`. Similarly, ANTsX

Table 1: Sampling of ANTsX functionality

<i>ANTsPy: Preprocessing</i>	
bias field correction	<code>n4_bias_field_correction(...)</code>
image denoising	<code>denoise_image(...)</code>
<i>ANTsPy: Registration</i>	
image registration	<code>registration(...)</code>
image transformation	<code>apply_transforms(...)</code>
template generation	<code>build_template(...)</code>
landmark registration	<code>fit_transform_to_paired_points(...)</code>
time-varying landmark reg.	<code>fit_time_varying_transform_to_point_sets(...)</code>
integrate velocity field	<code>integrate_velocity_field(...)</code>
invert displacement field	<code>invert_displacement_field(...)</code>
<i>ANTsPy: Segmentation</i>	
MRF-based segmentation	<code>atropos(...)</code>
Joint label fusion	<code>joint_label_fusion(...)</code>
diffeomorphic thickness	<code>kelly_kapowski(...)</code>
<i>ANTsPy: Miscellaneous</i>	
Regional intensity statistics	<code>label_stats(...)</code>
Regional shape measures	<code>label_geometry_measures(...)</code>
B-spline approximation	<code>fit_bspline_object_to_scattered_data(...)</code>
Visualize images and overlays	<code>plot(...)</code>
<i>ANTsPyNet: Mouse-specific</i>	
brain extraction	<code>mouse_brain_extraction(...modality="t2"...)</code> <code>mouse_brain_extraction(...modality="ex5"...)</code>
brain parcellation	<code>mouse_brain_parcellation(...)</code>
cortical thickness	<code>mouse_cortical_thickness(...)</code>
super resolution	<code>mouse_histology_super_resolution(...)</code>

ANTsX provides state-of-the-art functionality for processing biomedical image data. Such tools, including deep learning networks, support a variety of mapping-related tasks. A more comprehensive listing of ANTsX tools with self-contained R and Python examples is provided as a gist page on GitHub (<https://tinyurl.com/antsxtutorial>).

507 contains an implementation of a well-performing patch-based denoising technique⁶⁰ and is
508 also available as an image filter to the ITK community, `ants.denoise_image(...)`.

509 **4.1.2 Image registration**

510 The ANTs registration toolkit is a complex framework permitting highly tailored solutions
511 to pairwise image registration scenarios⁸⁴. It includes innovative transformation models
512 for biological modeling^{54,67} and has proven capable of excellent performance^{55,85}. Vari-
513 ous parameter sets targeting specific applications have been packaged with the different
514 ANTsX packages, specifically ANTs, ANTsPy, and ANTsR⁴⁶. In ANTsPy, the function
515 `ants.registration(...)` is used to register a pair of images or a pair of image sets where
516 `type_of_transform` is a user-specified option that invokes a specific parameter set. For ex-
517 ample `type_of_transform='antsRegistrationSyNQuick[s]'` encapsulates an oft-used pa-
518 rameter set for quick registration whereas `type_of_transform='antsRegistrationSyN[s]'`
519 is a more detailed alternative. Transforming images using the derived transforms is performed
520 via the `ants.apply_transforms(...)` function.

521 Initially, linear optimization is initialized with center of (intensity) mass alignment typically
522 followed by optimization of both rigid and affine transforms using the mutual information
523 similarity metric. This is followed by diffeomorphic deformable alignment using symmetric
524 normalization (SyN) with Gaussian⁵⁴ or B-spline regularization⁶⁷ where the forward trans-
525 form is invertible and differentiable. The similarity metric employed at this latter stage is
526 typically either neighborhood cross-correlation or mutual information. Note that these pa-
527 rameter sets are robust to input image type (e.g., light sheet fluorescence microscopy, Nissl
528 staining, and the various MRI modalities) and are adaptable to mouse image geometry and
529 scaling. Further details can be found in the various documentation sources for these ANTsX
530 packages.

531 **4.1.3 Template generation**

532 ANTsX provides functionality for constructing templates from a set (or multi-modal sets) of
533 input images as originally described⁵⁸ and recently used to create the DevCCF templates¹⁶.

534 An initial template estimate is constructed from an existing subject image or a voxelwise
535 average derived from a rigid pre-alignment of the image population. Pairwise registration
536 between each subject and the current template estimate is performed using the Symmetric
537 Normalization (SyN) algorithm⁵⁴. The template estimate is updated by warping all subjects
538 to the space of the template, performing a voxelwise average, and then performing a “shape
539 update” of this latter image by warping it by the average inverse deformation, thus yielding
540 a mean image of the population in terms of both intensity and shape. The corresponding
541 ANTsPy function is `ants.build_template(...)`.

542 4.1.4 Visualization

543 To complement the well-known visualization capabilities of R and Python, e.g., `ggplot2`
544 and `matplotlib`, respectively, image-specific visualization capabilities are available in the
545 `ants.plot(...)` function (Python). These are capable of illustrating multiple slices in
546 different orientations with other image overlays and label images.

547 4.2 Mapping fMOST data to AllenCCFv3

548 4.2.1 Preprocessing

- 549 • *Downsampling*. The first challenge when mapping fMOST images into the AllenCCFv3
550 is addressing the resolution scale of the data. Native fMOST data from an individual
551 specimen can range in the order of terabytes, which leads to two main problems. First,
552 volumetric registration methods (particularly those estimating local deformation) have
553 high computational complexity and typically cannot operate on such high-resolution
554 data under reasonable memory and runtime constraints. Second, the resolution of
555 the AllenCCFv3 atlas is much lower than the fMOST data, thus the mapping process
556 will cause much of the high-resolution information in the fMOST images to be lost
557 regardless. Thus, we perform a cubic B-spline downsampling of the fMOST data to
558 reduce the resolution of each image to match the isotropic $25 \mu\text{m}$ voxel resolution of the
559 AllenCCFv3 intensity atlas using `ants.resample_image(...)`. An important detail

560 to note is that while the fMOST images and atlas are downsampled, the mapping
561 learned during the registration is assumed to be continuous. Thus, after establishing
562 the mapping to the AllenCCFv3, we can interpolate the learned mapping and apply it
563 directly to the high-resolution native data directly to transform any spatially aligned
564 data (such as the single-cell neuron reconstructions) into the AllenCCFv3.

- 565 • *Stripe artifact removal.* Repetitive pattern artifacts are a common challenge in fMOST
566 imaging where inhomogeneity during the cutting and imaging of different sections can
567 leave stripes of hyper- and hypo-intensity across the image. These stripe artifacts
568 can be latched onto by the registration algorithm as unintended features that are
569 then misregistered to non-analogous structures in the AllenCCFv3. We address these
570 artifacts by fitting a 3-D bandstop (notch) filter to target the frequency of the stripe
571 patterns and removing them prior to the image registration.
- 572 • *Inhomogeneity correction.* Regional intensity inhomogeneity can also occur within
573 and between sections in fMOST imaging due to staining or lighting irregularity during
574 acquisition. Similar to stripe artifacts, intensity gradients due to inhomogeneity
575 can be misconstrued as features during the mapping and result in matching of non-
576 corresponding structures. Our pipeline addresses these intensity inhomogeneities using
577 N4 bias field correction⁶¹, `ants.n4_bias_field_correction(...)`.

578 4.2.2 Steps for spatial normalization to AllenCCFv3

- 579 1. *Average fMOST atlas as an intermediate target.* Due to the preparation of the mouse
580 brain for fMOST imaging, the resulting structure in the mouse brain has several large
581 morphological deviations from the AllenCCFv3 atlas. Most notable of these is an en-
582 largement of the ventricles, and compression of cortical structures. In addition, there is
583 poor intensity correspondence for the same anatomic features due to intensity dissim-
584 ilarity between imaging modalities. We have found that standard intensity-base reg-
585 istration is insufficient to capture the significant deformations required to map these
586 structures correctly into the AllenCCFv3. We address this challenge in ANTsX by
587 using explicitly corresponding parcellations of the brain, ventricles and surrounding

structures to directly recover these large morphological differences. However, generating these parcellations for each individual mouse brain is a labor-intensive task. Our solution is to create an average atlas whose mapping to AllenCCFv3 encapsulates these large morphological differences to serve as an intermediate registration point. This has the advantage of only needing to generate one set of corresponding annotations which is used to register between the two atlas spaces. New images are first aligned to the fMOST average atlas, which shares common intensity and morphological features and thus can be achieved through standard intensity-based registration.

2. *Average fMOST atlas construction.* An intensity and shape-based contralaterally symmetric average of the fMOST image data is constructed from 30 images and their contralateral flipped versions. We ran three iterations of the atlas construction using the default settings. Additional iterations (up to six) were evaluated and showed minimal changes to the final atlas construction, suggesting a convergence of the algorithm.

3. *fMOST atlas to AllenCCFv3 alignment.* Alignment between the fMOST average atlas and AllenCCFv3 was performed using a one-time annotation-driven approach. Label-to-label registration is used to align 7 corresponding annotations in both atlases in the following: 1) brain mask/ventricles, 2) caudate/putamen, 3) fimbria, 4) posterior choroid plexus, 5) optic chiasm, 6) anterior choroid plexus, and 7) habenular commissure. The alignments were performed sequentially, with the largest, most relevant structures being aligned first using coarse registration parameters, followed by other structures using finer parameters. This coarse-to-fine approach allows us to address large morphological differences (such as brain shape and ventricle expansion) at the start of registration and then progressively refine the mapping using the smaller structures. The overall ordering of these structures was determined manually by an expert anatomist, where anatomical misregistration after each step of the registration was evaluated and used to determine which structure should be used in the subsequent iteration to best improve the alignment. The transformation from this one-time expert-guided alignment is preserved and used as the canonical fMOST atlas to AllenCCFv3 mapping in the pipeline.

- 617 4. *Alignment of individual fMOST mouse brains.* The canonical transformation between
618 the fMOST atlas and AllenCCFv3 greatly simplifies the registration of new individ-
619 ual fMOST mouse brains into the AllenCCFv3. Each new image is first registered
620 into the fMOST average atlas, which shares intensity, modality, and morphologi-
621 cal characteristics. This allows us to leverage standard, intensity-based registration
622 functionality⁸⁴ available in ANTsX to perform this alignment. Transformations are
623 then concatenated to the original fMOST image to move it into the AllenCCFv3 space
624 using `ants.apply_transforms(...)`.
- 625 5. *Transformation of single cell neurons.* A key feature of fMOST imaging is the ability
626 to reconstruct and examine whole-brain single neuron projections⁷⁹. Spatial mapping
627 of these neurons from individual brains into the AllenCCFv3 allows investigators to
628 study different neuron types within the same space and characterize their morphology
629 with respect to their transcriptomics. Mappings found between the fMOST image
630 and the AllenCCFv3 using our pipeline can be applied in this way to fMOST neuron
631 reconstruction point set data using `ants.apply_transforms_to_points(..)`.

632 **4.3 Mapping MERFISH data to AllenCCFv3**

633 **4.3.1 Preprocessing**

- 634 • *Initial volume reconstruction.* Alignment of MERFISH data into a 3-D atlas space
635 requires an estimation of anatomical structure within the data. For each section,
636 this anatomic reference image was created by aggregating the number of detected
637 genetic markers (across all probes) within each pixel of a $10 \times 10 \mu\text{m}^2$ grid to match
638 the resolution of the $10 \mu\text{m}$ AllenCCFv3 atlas. These reference image sections are then
639 coarsely reoriented and aligned across sections using manual annotations of the most
640 dorsal and ventral points of the midline. The procedure produces an anatomic image
641 stack that serves as an initialization for further global mappings into the AllenCCFv3.
- 642 • *Anatomical correspondence labeling.* Mapping the MERFISH data into the AllenCCFv3
643 requires us to establish correspondence between the anatomy depicted in the MERFISH

and AllenCCFv3 data. Intensity-based features in MERFISH data are not sufficiently apparent to establish this correspondence, so we need to generate instead corresponding anatomical labelings of both images with which to drive registration. These labels are already available as part of the AllenCCFv3; thus, the main challenge is deriving analogous labels from the spatial transcriptomic maps of the MERFISH data. Toward this end, we assigned each cell from the scRNA-seq dataset to one of the following major regions: cerebellum, CTXsp, hindbrain, HPF, hypothalamus, isocortex, LSX, midbrain, OLF, PAL, sAMY, STRd, STRv, thalamus and hindbrain. A label map of each section was generated for each region by aggregating the cells assigned to that region within a $10 \times 10\mu m^2$ grid. The same approach was used to generate more fine grained region specific landmarks (i.e., cortical layers, habenula, IC). Unlike the broad labels which cover large swaths of the section these regions are highly specific to certain parts of the section. Once cells in the MERFISH data are labeled, morphological dilation is used to provide full regional labels for alignment into the AllenCCFv3.

- *Section matching.* Since the MERFISH data is acquired as sections, its 3-D orientation may not be fully accounted for during the volume reconstruction step, due to the particular cutting angle. This can lead to obliqueness artifacts in the section where certain structures can appear to be larger or smaller, or missing outright from the section. To address this, we first use a global alignment to match the orientations of the MERFISH sections to the atlas space. In our pipeline, this section matching is performed in the reverse direction by performing a global affine transformation of the AllenCCFv3 into the MERFISH data space, and then resampling digital sections from the AllenCCFv3 to match each MERFISH section. This approach limits the overall transformation and thus resampling that is applied to the MERFISH data, and, since the AllenCCFv3 is densely sampled, it also reduces in-plane artifacts that result from missing sections or undefined spacing in the MERFISH data.

670 **4.3.2 2.5D deformable, landmark-driven alignment to AllenCCFv3**

671 After global alignment of the AllenCCFv3 into the MERFISH dataset, 2D per-section de-
672 formable refinements are used to address local differences between the MERFISH sections
673 and the resampled AllenCCFv3 sections. Nine registrations were performed in sequence us-
674 ing a single label at each iteration in the following order: 1) brain mask, 2) isocortex (layer
675 2+3), 3) isocortex (layer 5), 4) isocortex (layer 6), 5) striatum, 6) medial habenula, 7) lateral
676 habenula, 8) thalamus, and 9) hippocampus. This ordering was determined empirically by
677 an expert anatomist who prioritized which structure to use in each iteration by evaluat-
678 ing the anatomical alignment from the previous iteration. Global and local mappings are
679 then all concatenated (with appropriate inversions) to create the final mapping between the
680 MERFISH data and AllenCCFv3. This mapping is then used to provide a point-to-point
681 correspondence between the original MERFISH coordinate space and the AllenCCFv3 space,
682 thus allowing mapping of individual genes and cell types located in the MERFISH data to
683 be directly mapped into the AllenCCFv3.

684 **4.4 DevCCF velocity flow transformation model**

685 Given multiple, linearly or non-linearly ordered point sets where individual points across the
686 sets are in one-to-one correspondence, we developed an approach for generating a velocity
687 flow transformation model to describe a time-varying diffeomorphic mapping as a variant of
688 the landmark matching solution. Integration of the resulting velocity field can then be used
689 to describe the displacement between any two time points within this time-parameterized
690 domain. Regularization of the sparse correspondence between point sets is performed using
691 a generalized B-spline scattered data approximation technique⁸³, also created by the ANTsX
692 developers and contributed to ITK.

693 **4.4.1 Velocity field optimization**

694 To apply this methodology to the developmental templates¹⁶, we coalesced the manual an-
695 notations of the developmental templates into 26 common anatomical regions (see Figure 3).

696 We then used these regions to generate invertible transformations between successive time
697 points. Specifically each label was used to create a pair of single region images resulting in 26
698 pairs of “source” and “target” images. The multiple image pairs were simultaneously used to
699 iteratively estimate a diffeomorphic pairwise transform. Given the seven atlases E11.5, E13.5,
700 E15.5, E18.5, P4, P14, and P56, this resulted in 6 sets of transforms between successive time
701 points. Approximately 10^6 points were randomly sampled labelwise in the P56 template
702 space and propagated to each successive atlas providing the point sets for constructing the
703 velocity flow model. Approximately 125 iterations resulted in a steady convergence based
704 on the average Euclidean norm between transformed point sets. Ten integration points were
705 used and point sets were distributed along the temporal dimension using a log transform for
706 a more evenly spaced sampling. For additional information a help menu is available for the
707 ANTsPy function `ants.fit_time_varying_transform_to_point_sets(...)`.

708 4.5 ANTsXNet mouse brain applications

709 4.5.1 General notes regarding deep learning training

710 All network-based approaches described below were implemented and organized in the
711 ANTsXNet libraries comprising Python (ANTsPyNet) and R (ANTsRNet) analogs using
712 the Keras/Tensorflow libraries available as open-source in ANTsX GitHub repositories.
713 For the various applications, both share the identically trained weights for mutual re-
714 producibility. For all GPU training, we used Python scripts for creating custom batch
715 generators which we maintain in a separate GitHub repository for public availability
716 (<https://github.com/ntustison/ANTsXNetTraining>). These scripts provide details such as
717 batch size, choice of loss function, and network parameters. In terms of GPU hardware, all
718 training was done on a DGX (GPUs: 4X Tesla V100, system memory: 256 GB LRDIMM
719 DDR4).

720 Data augmentation is crucial for generalizability and accuracy of the trained networks.
721 Intensity-based data augmentation consisted of randomly added noise (i.e., Gaussian, shot,
722 salt-and-pepper), simulated bias fields based on N4 bias field modeling, and histogram warp-

723 ing for mimicking well-known MRI intensity nonlinearities^{46,86}. These augmentation tech-
724 niques are available in ANTsXNet (only ANTsPyNet versions are listed with ANTsRNet
725 versions available) and include:

- 726 • image noise: `ants.add_noise_to_image(...)`,
- 727 • simulated bias field: `antspynet.simulate_bias_field(...)`, and
- 728 • nonlinear intensity warping: `antspynet.histogram_warp_image_intensities(...)`.

729 Shape-based data augmentation used both random linear and nonlinear deformations in
730 addition to anisotropic resampling in the three canonical orientations to mimic frequently
731 used acquisition protocols for mice brains:

- 732 • random spatial warping: `antspynet.randomly_transform_image_data(...)` and
- 733 • anisotropic resampling: `ants.resample_image(...)`.

734 4.5.2 Brain extraction

735 Similar to human neuroimage processing, brain extraction is a crucial preprocessing step
736 for accurate brain mapping. We developed similar functionality for T2-weighted mouse
737 brains. This network uses a conventional U-net architecture⁸⁷ and, in ANTsPyNet, this
738 functionality is available in the program `antspynet.mouse_brain_extraction(...)`.
739 For the two-shot T2-weighted brain extraction network, two brain templates were gen-
740 erated along with their masks. One of the templates was generated from orthogonal
741 multi-plane, high resolution data⁶⁹ which were combined to synthesize isotropic volu-
742 metric data using the B-spline fitting algorithm⁸³. This algorithm is encapsulated in
743 `ants.fit_bspline_object_to_scattered_data(...)` where the input is the set of voxel
744 intensity values and each associated physical location. Since each point can be assigned
745 a confidence weight, we use the normalized gradient value to more heavily weight edge
746 regions. Although both template/mask pairs are available in the GitHub repository
747 associated with this work, the synthesized volumetric B-spline T2-weighted pair is available
748 within ANTsXNet through the calls:

- 749 • template: `antspynet.get_antsxnet_data("bsplineT2MouseTemplate")` and
750 • mask: `antspynet.get_antsxnet_data("bsplineT2MouseTemplateBrainMask")`.

751 **4.5.3 Brain parcellation**

752 The T2-weighted brain parcellation network is also based on a 3-D U-net architecture and the
753 T2-w DevCCF P56 template component with extensive data augmentation, as described pre-
754 viously. Intensity differences between the template and any brain extracted input image are
755 minimized through the use of the rank intensity transform (`ants.rank_intensity(...)`).
756 Shape differences are reduced by the additional preprocessing step of warping the brain ex-
757 tracted input image to the template. Additional input channels include the prior probability
758 images created from the template parcellation. These images are also available through the
759 ANTsXNet `get_antsxnet_data(...)` interface.

760 **Data availability**

761 All data and software used in this work are publicly available. The DevCCF atlas is
762 available at <https://kimlab.io/brain-map/DevCCF/>. ANTsPy, ANTsR, ANTsPyNet, and
763 ANTsRNet are available through GitHub at the ANTsX Ecosystem (<https://github.com/ANTsX>). Training scripts for all deep learning functionality in ANTsXNet can also be
764 found on GitHub (<https://github.com/ntustison/ANTsXNetTraining>). A GitHub reposi-
765 tory specifically pertaining to the AllenCCFv3 mapping is available at <https://github.com/>
766 [dontminchenit/CCFAAlignmentToolkit](https://github.com/dontminchenit/CCFAAlignmentToolkit). For the other two contributions contained in this
767 work, the longitudinal DevCCF mapping and mouse cortical thickness pipeline, we refer the
768 interested reader to <https://github.com/ntustison/ANTsXMouseBrainMapping>.

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⁷⁷⁴ Author contributions

⁷⁷⁵ N.T., M.C., and J.G. wrote the main manuscript text and figures. M.C., M.K., R.D., S.S.,
⁷⁷⁶ Q.W., L.G., J.D., C.G., and J.G. developed the Allen registration pipelines. N.T. and F.K.
⁷⁷⁷ developed the time-varying velocity transformation model for the DevCCF. N.T. and M.T.
⁷⁷⁸ developed the brain parcellation and cortical thickness methodology. All authors reviewed
⁷⁷⁹ the manuscript.

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