

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

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<sup>23</sup> **Abstract**

<sup>24</sup> Large-scale, international collaborative efforts by members of the BRAIN Initiative Cell  
<sup>25</sup> Census Network (BICCN) consortium are aggregating the most comprehensive reference  
<sup>26</sup> database to date for diverse cell type profiling of the mouse brain, which encompasses over  
<sup>27</sup> 40 different multi-modal profiling techniques from more than 30 research groups. One cen-  
<sup>28</sup> tral challenge for this integrative effort has been the need to map these unique datasets into  
<sup>29</sup> common reference spaces such that the spatial, structural, and functional information from  
<sup>30</sup> different cell types can be jointly analyzed. However, significant variation in the acquisition,  
<sup>31</sup> tissue processing, and imaging techniques across data types makes mapping such diverse  
<sup>32</sup> data a multifarious problem. Different data types exhibit unique tissue distortion and sig-  
<sup>33</sup> nal characteristics that precludes a single mapping strategy from being generally applicable  
<sup>34</sup> across all cell type data. Tailored mapping approaches are often needed to address the unique  
<sup>35</sup> barriers present in each modality. This work highlights modular atlas mapping strategies  
<sup>36</sup> developed across separate BICCN studies using the Advanced Normalization Tools Ecosys-  
<sup>37</sup> tem (ANTsX) to map spatial transcriptomic (MERFISH) and high-resolution morphology  
<sup>38</sup> (fMOST) mouse brain data into the Allen Common Coordinate Framework (AllenCCFv3),  
<sup>39</sup> and developmental (MRI and LSFM) data into the Developmental Common Coordinate  
<sup>40</sup> Framework (DevCCF). We discuss common mapping strategies that can be shared across  
<sup>41</sup> modalities and driven by specific challenges from each data type. These mapping strategies  
<sup>42</sup> include novel open-source contributions that are made publicly available through ANTSX.  
<sup>43</sup> These include 1) a velocity flow-based approach for continuously mapping developmental  
<sup>44</sup> trajectories such as that characterizing the DevCCF and 2) an automated framework for de-  
<sup>45</sup> termining structural morphology solely through the leveraging of publicly resources. Finally,  
<sup>46</sup> we provide general guidance to aid investigators to tailor these strategies to address unique  
<sup>47</sup> data challenges without the need to develop additional specialized software.

## <sup>48</sup> 1 Introduction

<sup>49</sup> Over the past decade there have been significant advancements in mesoscopic single-cell analysis of the mouse brain. It is now possible to track single neurons in mouse brains<sup>1</sup>, observe whole brain developmental changes on a cellular level<sup>2</sup>, associate brain regions and tissues with their genetic composition<sup>3</sup>, and locally characterize neural connectivity<sup>4</sup>. Much of these scientific achievements have been made possible due to breakthroughs in high resolution cell profiling and imaging techniques that permit submicron, multi-modal, 3-D characterizations of whole mouse brains. Among these include advanced techniques such as micro-optical sectioning tomography<sup>6</sup>, tissue clearing<sup>1,7</sup>, spatial transcriptomics<sup>9</sup>, and single-cell genomic profiling<sup>10</sup>, which have greatly expanded the resolution and specificity of single-cell measurements in the brain.

<sup>59</sup> Recent efforts by the National Institutes of Health's Brain Research Through Advancing Innovative Neurotechnologies (BRAIN) Initiative has pushed for large-scale, international collaborative efforts to utilize these advanced single-cell techniques to create a comprehensive reference database for high-resolution transcriptomic, epigenomic, structural and imaging data of the mouse brain. This consortium of laboratories and data centers, known as the BRAIN Initiative Cell Census Network (BICCN), has archived datasets encompassing over 40 different multi-modal profiling techniques from more than 30 research groups, each providing unique characterizations of distinct cell types in the brain<sup>11</sup>. Several of these modalities have been further developed into reference atlases to facilitate spatial alignment of individual brains and different data types into a common coordinate framework (CCF), thus allowing diverse single-cell information to be analyzed in an integrated manner. The most notable of these atlases is the Allen Mouse Brain Common Coordinate Framework (AllenCCFv3)<sup>12</sup>, which serves as a primary target coordinate space for much of the work associated with the BICCN. Other atlases include modality-specific atlases<sup>13–15</sup>, and spatiotemporal atlases<sup>16,17</sup> for the developing mouse brain.

<sup>74</sup> **1.1 Mouse brain mapping**

<sup>75</sup> The cross-modality associations that can be learned from mapping different cell type data  
<sup>76</sup> into a CCF is critical for improving our understanding of the complex relationships between  
<sup>77</sup> cellular structure, morphology, and genetics in the brain. However, finding an accurate map-  
<sup>78</sup> ping between each individual mouse brain and a CCF is a challenging and heterogeneous  
<sup>79</sup> task. There is significant variance in the imaging protocols across different cell type data  
<sup>80</sup> as well as different tissue processing and imaging methods which can potentially introduce  
<sup>81</sup> tissue distortion and signal differences<sup>18,19</sup>. Certain modalities can have poor intensity corre-  
<sup>82</sup> spondence with the CCF, negatively impacting image alignment accuracy. Studies targeting  
<sup>83</sup> specific regions or cell types can lead to missing anatomical correspondences. Other consid-  
<sup>84</sup> erations include artifacts such as tissue distortion, holes, bubbles, folding, tears, and missing  
<sup>85</sup> sections in the data that often require manual correction<sup>20-23</sup>. Given the diversity of these  
<sup>86</sup> challenges, it is unlikely any single mapping approach can be generally applicable across all  
<sup>87</sup> cell type data. Diverse, and often specialized, strategies are needed to address the unique  
<sup>88</sup> barriers present for mapping each modality.

<sup>89</sup> Existing solutions to address mapping cell type data into the AllenCCFv3 falls broadly into  
<sup>90</sup> three main categories. The first consists of integrated processing platforms that directly  
<sup>91</sup> provide mapped data to the users. These include the Allen Brain Cell Atlas<sup>24</sup> for the Allen  
<sup>92</sup> Reference Atlas (ARA) and associated data, the Brain Architecture Portal<sup>25</sup> for combined ex  
<sup>93</sup> vivo radiology and histology data, OpenBrainMap<sup>26</sup> for connectivity data, and the Image and  
<sup>94</sup> Multi-Morphology Pipeline<sup>27</sup> for high resolution morphology data. These platforms provide  
<sup>95</sup> users online access to pre-processed, multi-modal cell type data that are already mapped to  
<sup>96</sup> the AllenCCFv3. The platforms are designed such that the data is interactively manipulated  
<sup>97</sup> by users through integrated visualization software that allow users to spatially manipulate  
<sup>98</sup> and explore each dataset within the mapped space. While highly convenient for investigators  
<sup>99</sup> who are interested in studying the specific modalities provided by these platforms, these  
<sup>100</sup> systems can be limited in flexibility, general applicability, and public availability. As a  
<sup>101</sup> result, investigators often find it difficult to apply the same mapping solutions to their own  
<sup>102</sup> data.

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

115 The last category consist of modular mapping approaches constructed using general im-  
116 age analysis toolkits, which are software packages that include modular image processing,  
117 segmentation and registration tools that have been previously developed, and validated for  
118 multiple application areas. Examples of such toolkits include elastix<sup>44</sup>, Slicer3D<sup>45</sup>, ANTsX<sup>46</sup>,  
119 and several others which have all been applied towards mouse brain spatial mapping. The  
120 main challenge, in these mouse-specific study scenarios, is that tailored pipelines often need  
121 be constructed from available software components. Investigators must therefore be familiar  
122 with the these tools for formulating new or adapting existing pipelines. However, in com-  
123 parison to previously described specialized mapping approaches, these approaches are often  
124 easier to create and prone to robustness, being typically constructed from pipeline compo-  
125 nents which have been previously vetted in other contexts. In this work, we highlight such  
126 mapping strategies designed using the ANTsX framework to map distinct mouse cell type  
127 data with different characteristics into existing CCFs.

## 128 1.2 Advanced Normalization Tools (ANTsX)

129 The Advanced Normalization Tools Ecosystem (ANTsX) has been used in a number of  
130 applications for mapping mouse brain data as part of core processing steps in various

<sup>131</sup> workflows<sup>30,47–50</sup>, particularly its pairwise, intensity-based image registration capabilities<sup>51</sup>  
<sup>132</sup> and bias field correction<sup>52</sup>. Historically, ANTsX development is originally based on fun-  
<sup>133</sup> damental approaches to image mapping<sup>53–55</sup>, particularly in the human brain, which has  
<sup>134</sup> resulted in core contributions to the field such as the widely-used Symmetric Normalization  
<sup>135</sup> (SyN) algorithm<sup>51</sup>. Since its development, various independent platforms have been used  
<sup>136</sup> to evaluate ANTsX image registration capabilities in the context of different application  
<sup>137</sup> foci which include multi-site brain MRI data<sup>56</sup>, pulmonary CT data<sup>57</sup>, and most recently,  
<sup>138</sup> multi-modal brain registration in the presence of tumors<sup>58</sup>.

<sup>139</sup> Apart from its registration capabilities, ANTsX comprises additional functionality such  
<sup>140</sup> as template generation<sup>59</sup>, intensity-based segmentation<sup>60</sup>, preprocessing<sup>52,61</sup>, deep learning  
<sup>141</sup> networks<sup>46</sup>, and other utilities relevant to brain mapping (see Table 1). The use of the toolkit  
<sup>142</sup> has demonstrated high performance in multiple application areas (e.g., consensus labeling<sup>62</sup>,  
<sup>143</sup> brain tumor segmentation<sup>63</sup>, and cardiac motion estimation<sup>64</sup>). Importantly, ANTsX is built  
<sup>144</sup> on the Insight Toolkit (ITK)<sup>65</sup> deriving benefit from the open-source community of scientists  
<sup>145</sup> and programmers as well as providing an important resource for algorithmic development,  
<sup>146</sup> evaluation, and improvement.

<sup>147</sup> With respect to mouse cell type data, ANTsX provides a comprehensive toolset which serves  
<sup>148</sup> as a basis for developing modular frameworks for mapping diverse image data into com-  
<sup>149</sup> mon coordinate frameworks (CCFs). Herein, we highlight its application for mapping data  
<sup>150</sup> from separate BICCN projects focused on distinct data types: morphology data using flu-  
<sup>151</sup> orescence micro-optical sectioning tomography (fMOST), spatial transcriptomics from mul-  
<sup>152</sup> tiplexed error-robust fluorescence in situ hybridization (MERFISH) data, and time-series  
<sup>153</sup> developmental data using light sheet fluorescence microscopy (LSFM) and magnetic reso-  
<sup>154</sup> nance imaging (MRI). We describe both shared and targeted strategies developed to address  
<sup>155</sup> the specific challenges of these modalities.

### <sup>156</sup> 1.3 Novel ANTsX-based open-source contributions

<sup>157</sup> We introduce two novel inclusions to the ANTsX toolset that were developed as part of  
<sup>158</sup> the MRI mapping and analysis pipeline for the Developmental Common Coordinate Frame-

<sup>159</sup> work (DevCCF). Consistent with previous ANTsX development, newly introduced capa-  
<sup>160</sup> bilities introduced below are available through ANTsX (specifically, via R and Python  
<sup>161</sup> ANTsX packages), and illustrated through self-contained examples in the ANTsX tuto-  
<sup>162</sup> rial (<https://tinyurl.com/antsxtutorial>) with a dedicated GitHub repository specific to this  
<sup>163</sup> work (<https://github.com/ntustison/ANTsXMouseBrainMapping>). To complement stan-  
<sup>164</sup> dard preprocessing steps (e.g., bias correction, brain masking), additional mouse brain spe-  
<sup>165</sup> cific tools have also been introduced to the ANTsX ecosystem, such as section reconstruction  
<sup>166</sup> and landmark-based alignment with corresponding processing scripts (<https://github.com/>  
<sup>167</sup> [dontminchenit/CCFAAlignmentToolkit](https://github.com/dontminchenit/CCFAAlignmentToolkit)).

<sup>168</sup> **1.3.1 Continuously mapping the DevCCF developmental trajectory with a ve-**  
<sup>169</sup> **locity flow model**

<sup>170</sup> Recently, the Developmental Common Coordinate Framework (DevCCF) was introduced to  
<sup>171</sup> the mouse brain research community as a public resource<sup>16</sup> comprising symmetric atlases of  
<sup>172</sup> multi-modal image data and anatomical segmentations defined by developmental ontology.  
<sup>173</sup> These templates sample the mouse embryonic days E11.5, E13.5, E15.5, E18.5 and postnatal  
<sup>174</sup> days P4, P14, and P56. Modalities include LSFM and at least four MRI contrasts per  
<sup>175</sup> developmental stage. Anatomical parcellations are also available for each time point and  
<sup>176</sup> were generated from ANTsX-based mappings of gene expression and other cell type data.  
<sup>177</sup> Additionally, the P56 template was integrated with the AllenCCFv3 to further enhance the  
<sup>178</sup> practical utility of the DevCCF. These processes, specifically template generation and multi-  
<sup>179</sup> modal image mapping, were performed using ANTsX functionality in the presence of image  
<sup>180</sup> mapping difficulties such as missing data and tissue distortion.

<sup>181</sup> Given the temporal gaps in the discrete set of developmental atlases, we also provide an  
<sup>182</sup> open-source framework for inferring correspondence within the temporally continuous do-  
<sup>183</sup> main sampled by the existing set of embryonic and postnatal atlases of the DevCCF. This  
<sup>184</sup> recently developed functionality permits the generation of a diffeomorphic velocity flow trans-  
<sup>185</sup> formation model<sup>66</sup>, influenced by previous work<sup>67</sup>. The resulting time-parameterized veloc-  
<sup>186</sup> ity field spans the stages of the DevCCF where mappings between any two continuous time

<sup>187</sup> points within the span bounded by the E11.5 and P56 atlases are determined by numerical  
<sup>188</sup> integration of the optimized velocity field.

<sup>189</sup> **1.3.2 Automated structural parcellations of the mouse brain**

<sup>190</sup> In contrast to the pipeline development in human data<sup>46</sup>, limited tools exist yet to cre-  
<sup>191</sup> ate adequate training data for automated parcellations of the mouse brain. In addition,  
<sup>192</sup> mouse brain data acquisition often has unique issues, such as lower data quality or sampling  
<sup>193</sup> anisotropy which limits its applicability to high resolution resources (e.g., AllenCCFv3, De-  
<sup>194</sup> vCCF), specifically with respect to the corresponding granular brain parcellations derived  
<sup>195</sup> from numerous hours of expert annotation leveraging multi-modal imaging resources.

<sup>196</sup> Herein, we introduce a mouse brain parcellation pipeline for multi-modal MRI comprising  
<sup>197</sup> two novel deep learning components: two-shot learning brain extraction from data augmen-  
<sup>198</sup> tation of two ANTsX templates generated from two open datasets<sup>68,69</sup> and single-shot brain  
<sup>199</sup> parcellation derived from the AllenCCFv3 labelings mapped to the corresponding DevCCF  
<sup>200</sup> P56 template.

<sup>201</sup> Although we anticipate that this pipeline will be beneficial to the research community, this  
<sup>202</sup> work demonstrates more generally how one can leverage ANTsX tools and other public  
<sup>203</sup> resources for developing quantitative mouse brain morphological tools. Evaluation is per-  
<sup>204</sup> formed on an independent open dataset<sup>70</sup> comprising longitudinal acquisitions of multiple  
<sup>205</sup> specimens.

206 **2 Results**

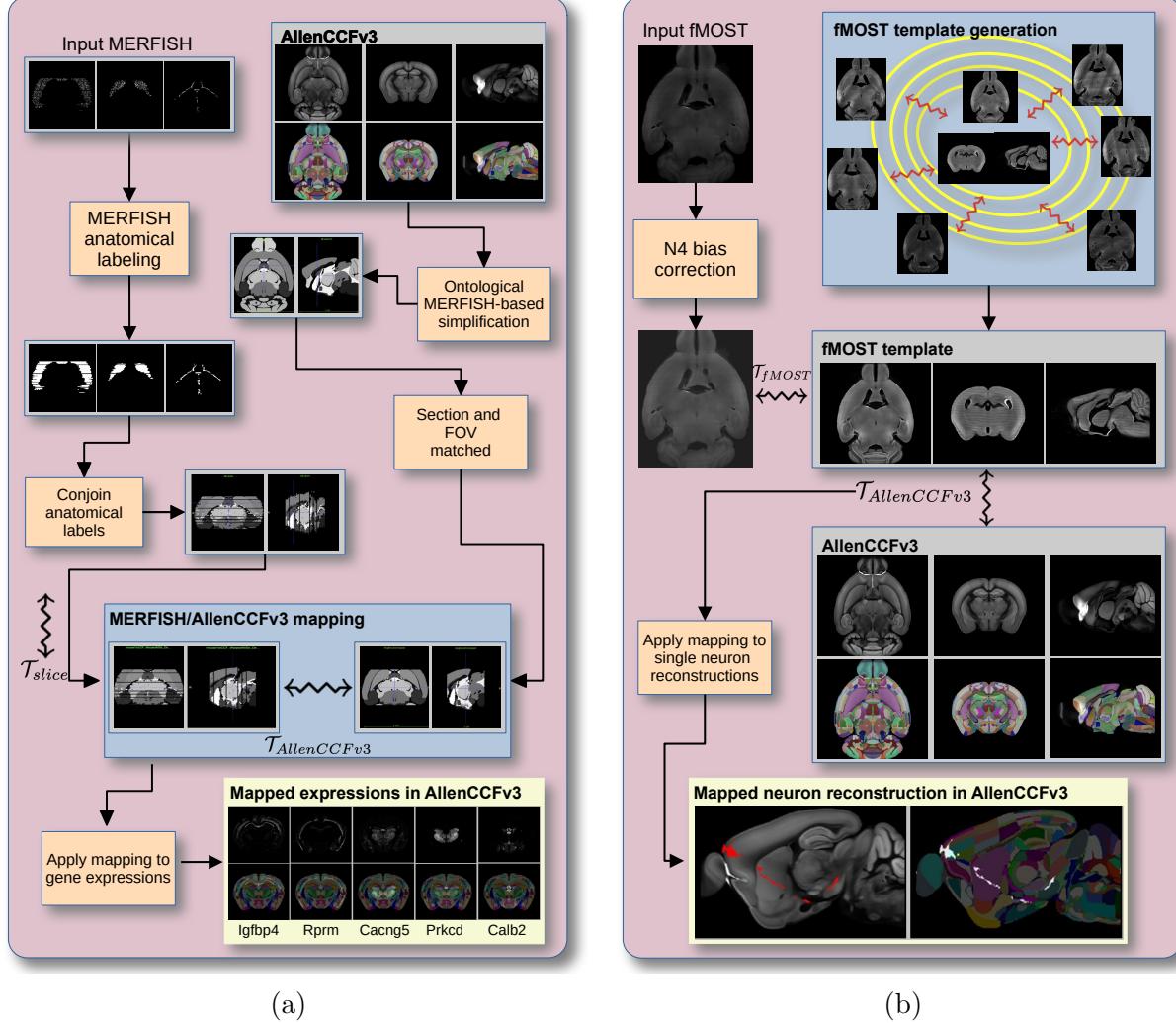


Figure 1: Diagram of the two ANTsX-based pipelines for mapping (a) MERFISH and (b)fMOST data into the space of AllenCCFv3. Each generates the requisite transforms,  $\mathcal{T}$ , to map individual images to the CCF.

207 **2.1 AllenCCFv3 brain image mapping**

208 **2.1.1 Mapping multiplexed error-robust fluorescence *in situ* hybridization  
209 (MERFISH) data**

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

212 data onto the AllenCCFv3 (see Figure 1(a)). This pipeline, used recently in creating a  
213 high-resolution transcriptomic atlas of the mouse brain<sup>50</sup>, performs mappings by first gen-  
214 erating anatomical labels from tissue related gene expressions in the MERFISH data, and  
215 then spatially matching these labels to corresponding anatomical tissue parcellations in the  
216 AllenCCFv3. The pipeline consists of MERFISH data specific preprocessing which includes  
217 section reconstruction, mapping corresponding anatomical labels between AllenCCFv3 and  
218 the spatial transcriptomic maps of the MERFISH data, and matching MERFISH sections  
219 to the atlas space. Following preprocessing, two main alignment steps were performed: 1)  
220 3-D global affine mapping and section matching of the AllenCCFv3 into the MERFISH data  
221 and 2) 2-D global and deformable mapping between each MERFISH section and matched  
222 AllenCCFv3 section. Mappings learned via each step in the pipeline are preserved and con-  
223 catenated to provide point-to-point correspondence between the original MERFISH data  
224 and AllenCCFv3, thus allowing individual gene expressions to be transferred into the Al-  
225 lenCCFv3.

226 **Data.** MERFISH mouse brain data was acquired using a previously detailed procedure<sup>50</sup>.  
227 Briefly, a brain of C57BL/6 mouse was dissected according to standard procedures and  
228 placed into an optimal cutting temperature (OCT) compound (Sakura FineTek 4583) in  
229 which it was stored at -80°C. The fresh frozen brain was sectioned at 10 $\mu m$  on Leica 3050  
230 S cryostats at intervals of 200 $\mu m$  to evenly cover the brain. A set of 500 genes were imaged  
231 that had been carefully chosen to distinguish the ~5200 clusters of our existing RNAseq  
232 taxonomy. For staining the tissue with MERFISH probes, a modified version of instructions  
233 provided by the manufacturer was used<sup>50</sup>. Raw MERSCOPE data were decoded using  
234 Vizgen software (v231). Cells were segmented based on DAPI and PolyT staining using  
235 Cellpose<sup>71,72</sup>. Segmentation was performed on a median z-plane (fourth out of seven) and  
236 cell borders were propagated to z-planes above and below. To assign cluster identity to each  
237 cell in the MERFISH dataset, we mapped the MERFISH cells to the scRNA-seq reference  
238 taxonomy.

239 **Evaluation.** Alignment of the MERFISH data into the AllenCCFv3 was qualitatively as-  
240 sessed by an expert anatomist at each iteration of the registration using known correspon-

<sup>241</sup> dence of gene markers and their associations with the AllenCCFv3. As previously reported<sup>50</sup>,  
<sup>242</sup> further assessment of the alignment showed that, of the 554 terminal regions (gray matter  
<sup>243</sup> only) in the AllenCCFv3, only seven small subregions were missed from the MERFISH  
<sup>244</sup> dataset: frontal pole, layer 1 (FRP1), FRP2/3, FRP5; accessory olfactory bulb, glomerular  
<sup>245</sup> layer (AOBgl); accessory olfactory bulb, granular layer (AOBgr); accessory olfactory bulb,  
<sup>246</sup> mitral layer (AOBmi); and accessory supraoptic group (ASO).

### <sup>247</sup> 2.1.2 Mapping fluorescence micro-optical sectioning tomography (fMOST) data

<sup>248</sup> **Overview.** We developed a pipeline for mapping fluorescence micro-optical sectioning to-  
<sup>249</sup> mography (fMOST) mouse brain images into the AllenCCFv3 (see Figure 1(b)). The pipeline  
<sup>250</sup> is adapted from previously developed frameworks for human brain mapping<sup>59</sup>, and uses a  
<sup>251</sup> modality specific (fMOST) average atlas to assist in the image registration and mapping.  
<sup>252</sup> This approach has been well validated in human studies<sup>73–75</sup>, and successfully used in other  
<sup>253</sup> mouse data<sup>12,15,34</sup>. Briefly, we construct an intensity- and shape-based average fMOST atlas  
<sup>254</sup> using 30 fMOST images to serve as an intermediate registration target for mapping fMOST  
<sup>255</sup> images from individual specimens into the AllenCCFv3. Preprocessing steps include down-  
<sup>256</sup> sampling to match the  $25\mu m$  isotropic AllenCCFv3, acquisition-based stripe artifact removal,  
<sup>257</sup> and inhomogeneity correction<sup>52</sup>. Preprocessing also includes a single annotation-driven reg-  
<sup>258</sup> istration to establish a canonical mapping between the fMOST atlas and the AllenCCFv3.  
<sup>259</sup> This step allows us to align expert determined landmarks to accurately map structures  
<sup>260</sup> with large morphological differences between the modalities, which are difficult to address  
<sup>261</sup> using standard approaches. Once this canonical mapping is established, standard intensity-  
<sup>262</sup> based registration is used to align each new fMOST image to the fMOST specific atlas.  
<sup>263</sup> This mapping is concatenated with the canonical fMOST atlas-to-AllenCCFv3 mapping to  
<sup>264</sup> further map each individual brain into the latter without the need to generate additional  
<sup>265</sup> landmarks. Transformations learned through this mapping can be applied to single neuron  
<sup>266</sup> reconstructions from the fMOST images to evaluate neuronal distributions across different  
<sup>267</sup> specimens into the AllenCCFv3 for the purpose of cell census analyses.

<sup>268</sup> **Data.** The high-throughput and high-resolution fluorescence micro-optical sectioning to-

269 mography (fMOST)<sup>76,77</sup> platform was used to image 55 mouse brains containing gene-defined  
270 neuron populations, with sparse transgenic expression<sup>78,79</sup>. In short, the fMOST imaging  
271 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  
272 imaging system where the green channel displays the green fluorescent protein (GFP) labeled  
273 neuron morphology and the red channel is used to visualize the counterstained propidium  
274 iodide cytoarchitecture. The spatial normalizations described in this work were performed  
275 using the red channel, which offered higher tissue contrast for alignment, although other  
276 approaches are possible including multi-channel registration.

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

## 284 2.2 Continuously mapping the DevCCF developmental trajectory 285 with a velocity flow model

286 The DevCCF is an openly accessible resource for the mouse brain research community<sup>16</sup>. It  
287 consists of multi-modal MRI and LSFM symmetric ANTsX-generated templates<sup>59</sup> sampling  
288 the mouse brain developmental trajectory, specifically the embryonic (E) and postnatal (P)  
289 days E11.5, E13.5, E15.5, E18.5 P4, P14, and P56. Each template space includes structural  
290 labels defined by a developmental ontology. Its utility is also enhanced by a coordinated  
291 construction with AllenCCFv3. Although this work represents a significant contribution,  
292 the gaps between time points potentially limit its applicability which could be addressed  
293 through the development of the ability to map not only between time points but also within  
294 and across time points.

295 To continuously generate transformations between the different stages of the DevCCF atlases,

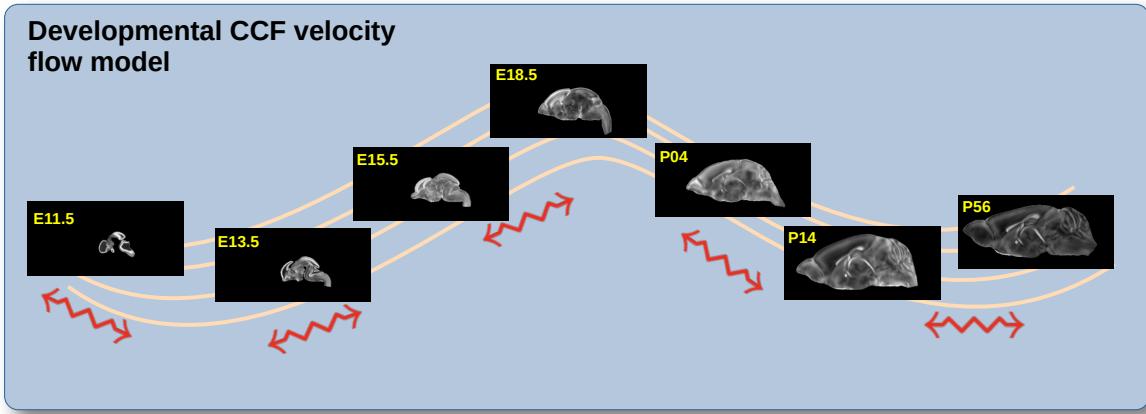


Figure 2: The spatial transformation between any two time points within the continuous DevCCF longitudinal developmental trajectory is available through the use of ANTsX functionality for generating a velocity flow model.

we developed a general velocity flow model approach which we apply to DevCCF-derived data. We also introduce this functionality into both the ANTsR and ANTsPy packages (for the latter, see `ants.fit_time_varying_transform_to_point_sets(...)`) for potential application to this and other analogous scenarios (e.g., modeling the cardiac and respiratory cycles). 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

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

Prior to velocity field optimization, all data were rigidly transformed to DevCCF P56 using the centroids of the common label sets. In order to determine the landmark correspondence

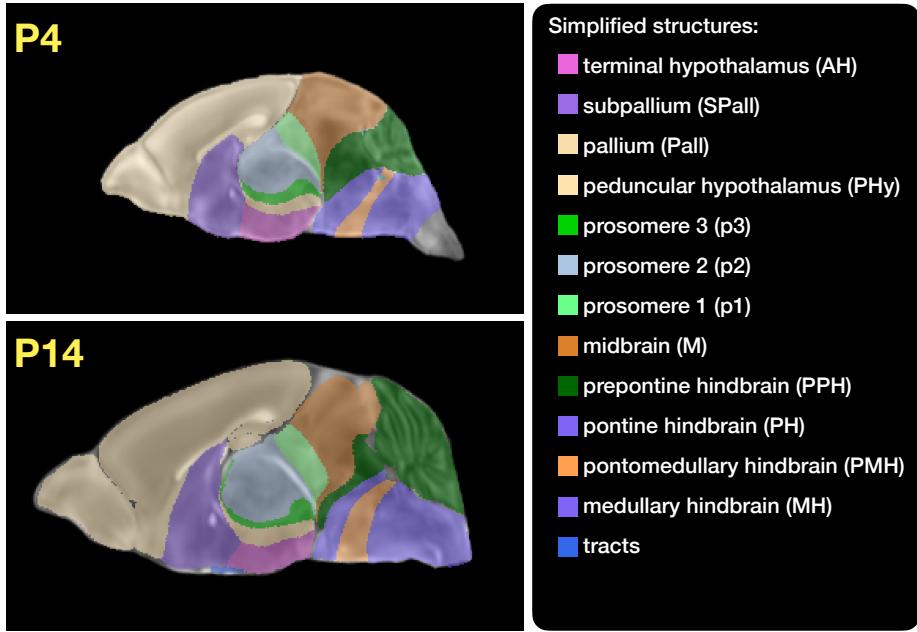


Figure 3: Annotated regions representing common labels across developmental stages which are illustrated for both P4 and P14.

312 across DevCCF stages, the multi-metric capabilities of `ants.registration(...)` were used.  
 313 Instead of performing intensity-based pairwise registration directly on these multi-label im-  
 314 ages, each label was used to construct a separate fixed and moving image pair resulting in a  
 315 multi-metric registration optimization scenario involving 24 binary image pairs (each label  
 316 weighted equally) for optimizing diffeomorphic correspondence between neighboring time  
 317 point atlases using the mean squares metric and the symmetric normalization transform<sup>51</sup>.  
 318 To generate the set of common point sets across all seven developmental atlases, the label  
 319 boundaries and whole regions were sampled in the P56 atlas and then propagated to each  
 320 atlas using the transformations derived from the pairwise registrations. We selected a sam-  
 321 pling rate of 10% for the contour points and 1% for the regional points for a total number  
 322 of points being per atlas being 173303 ( $N_{contour} = 98151$  and  $N_{region} = 75152$ ). Regional  
 323 boundary points were weighted twice as those of non-boundary points during optimization.

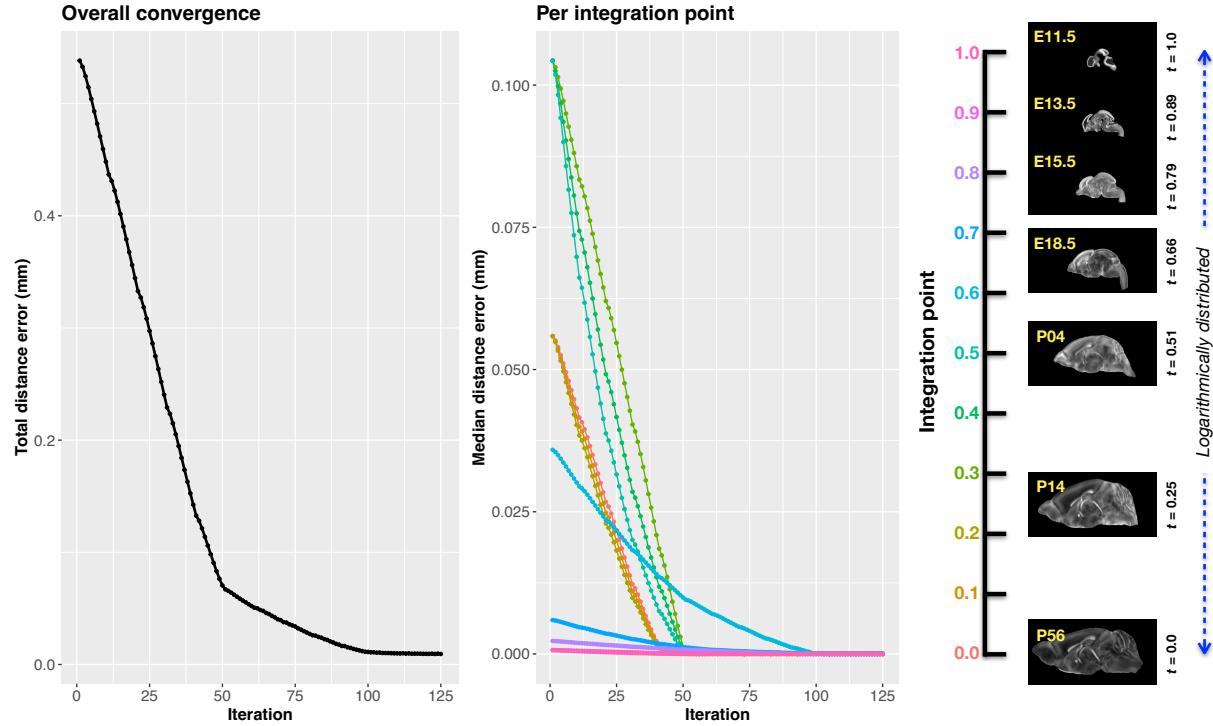


Figure 4: Convergence of the optimization of the velocity field for describing the transformation through the developmental stages from E11.5 through P56. Integration points in diagram on the right are color-coordinated with the center plot and placed in relation to the logarithmically situated temporal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

### <sup>324</sup> 2.2.2 Velocity field optimization

<sup>325</sup> The velocity field was optimized using the input composed of the seven corresponding point  
<sup>326</sup> sets and their associated weight values, the selected number of integration points for the  
<sup>327</sup> velocity field ( $N = 11$ ), and the parameters defining the geometry of the spatial dimensions  
<sup>328</sup> of the velocity field. Thus, the optimized velocity field described here is of size [256, 182, 360]  
<sup>329</sup> ( $50\mu\text{m}$  isotropic)  $\times 11$  integration points for a total compressed size of a little over 2 GB.

<sup>330</sup> This choice represented weighing the trade-off between tractability, portability, and accuracy.  
<sup>331</sup> However, all data and code to reproduce the results described are available in the dedicated  
<sup>332</sup> GitHub repository.

<sup>333</sup> The normalized time point scalar value for each atlas/point-set in the temporal domains  $[0, 1]$   
<sup>334</sup> was also defined. Given the increasingly larger gaps in the postnatal time point sampling,  
<sup>335</sup> we made two adjustments. Based on known mouse brain development, we used 28 days for

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

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

### 351 2.2.3 The velocity flow transformation model

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

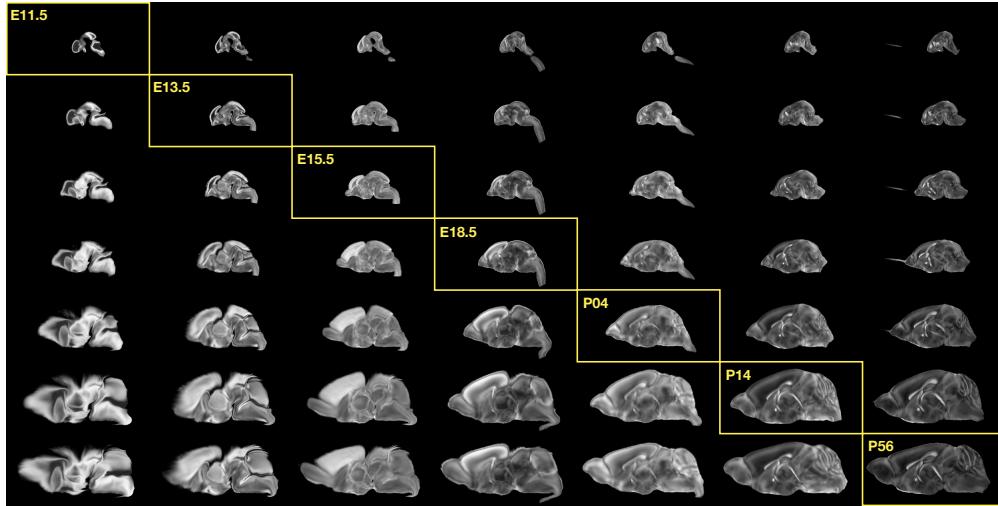


Figure 5: Mid-sagittal visualization of the effects of the transformation model in warping every developmental stage to the time point of every other developmental stage. The original images are located along the diagonal. Columns correspond to the warped original image whereas the rows represent the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

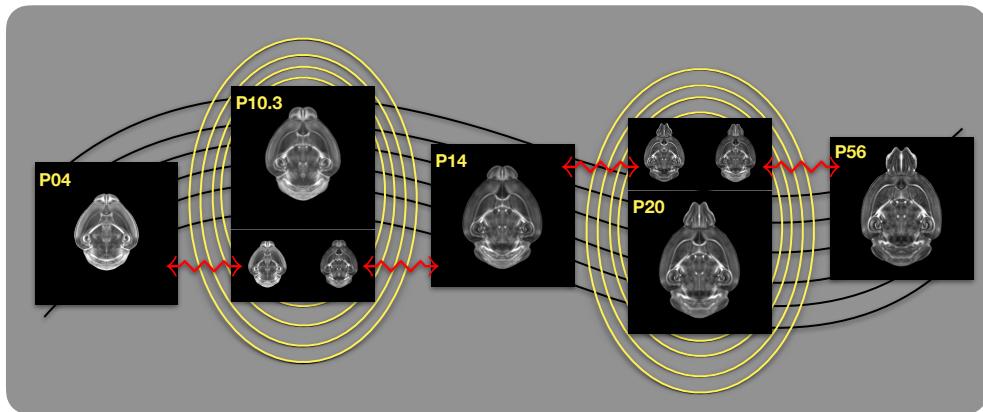


Figure 6: Illustration of the use of the velocity flow model for creating virtual templates at continuous time points not represented in one of the existing DevCCF time points. For example, FA templates at time point P10.3 and P20 can be generated by warping the existing temporally adjacent developmental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

### **363 2.3 Automated structural parcellations of the mouse brain**

**364** Brain parcellation strategies for the mouse brain are pivotal for understanding the complex  
**365** organization and function of murine nervous system<sup>80</sup>. By dividing the brain into distinct  
**366** regions based on anatomical, physiological, or functional characteristics, researchers can  
**367** investigate specific areas in isolation and identify their roles in various behaviors and pro-

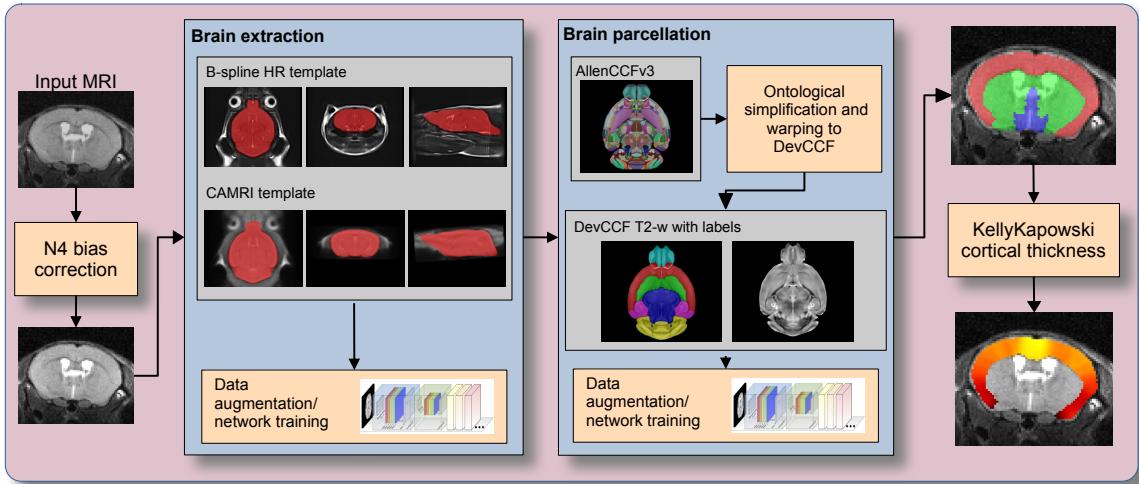


Figure 7: The mouse brain cortical parcellation pipeline integrating two deep learning components for brain extraction and brain parcellation prior to estimating cortical labels. Both deep learning networks rely heavily on aggressive data augmentation on templates built from open data and provide an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

cesses. For example, such parcellation schemes can help elucidate the spatial distribution of gene expression patterns<sup>81</sup> as well as identify functional regions involved in specific cognitive tasks<sup>82</sup>.

Although deep learning techniques have been used to develop useful parcellation tools for human brain research (e.g., SynthSeg<sup>83</sup>, ANTsXNet<sup>46</sup>), analogous development for the mouse brain is limited. In addition, mouse data is often characterized by unique imaging issues such as extreme anisotropic sampling which are often in sharp contrast to the high resolution template-based resources available within the community, e.g., AllenCCFv3 and DevCCF. We demonstrate how one can use the ANTsX tools to develop a complete mouse brain structural morphology pipeline as illustrated in Figure 7 and detailed below.

### 2.3.1 Two-shot mouse brain extraction network

In order to create a generalized mouse brain extraction network, we built whole-head templates from two publicly available datasets. The Center for Animal MRI (CAMRI) dataset<sup>68</sup> from the University of North Carolina at Chapel Hill consists of 16 T2-w MRI volumes of

<sup>382</sup> voxel resolution  $0.16 \times 0.16 \times 0.16\text{mm}^3$ . The second high-resolution dataset<sup>69</sup> comprises  
<sup>383</sup> 88 specimens each with three spatially aligned canonical views with in-plane resolution of  
<sup>384</sup>  $0.08 \times 0.08\text{mm}^2$  with a slice thickness of  $0.5\text{mm}$ . These three orthogonal views were used  
<sup>385</sup> to reconstruct a single high-resolution volume per subject using a B-spline fitting algorithm  
<sup>386</sup> available in ANTsX<sup>84</sup>. From these two datasets, two symmetric isotropic ANTsX templates<sup>59</sup>  
<sup>387</sup> were generated analogous to the publicly available ANTsX human brain templates used in  
<sup>388</sup> previous research<sup>85</sup>. Bias field simulation, intensity histogram warping, noise simulation,  
<sup>389</sup> random translation and warping, and random anisotropic resampling in the three canonical  
<sup>390</sup> directions were used for data augmentation in training a T2-w brain extraction network.

### <sup>391</sup> 2.3.2 Single-shot mouse brain parcellation network

<sup>392</sup> AllenCCFv3 and its hierarchical ontological labeling, along with the DevCCF, provides the  
<sup>393</sup> necessary data for developing a tailored structural parcellation network for multi-modal  
<sup>394</sup> imaging. The `allensdk` Python library permits the creation of any gross parcellation based  
<sup>395</sup> on the AllenCCFv3 ontology. For example, using `allensdk` we coalesced the labels to the  
<sup>396</sup> following six major structures: cerebral cortex, cerebral nuclei, brain stem, cerebellum, main  
<sup>397</sup> olfactory bulb, and hippocampal formation. This labeling was mapped to the P56 component  
<sup>398</sup> of the DevCCF for use with the T2-w template component. Both the T2-w P56 DevCCF  
<sup>399</sup> and labelings, in conjunction with the data augmentation described previously for brain  
<sup>400</sup> extraction, were used to train the proposed brain parcellation network. Note that other  
<sup>401</sup> brain parcellation networks have also been trained using alternative regions and parcellation  
<sup>402</sup> schemes and are available in the same ANTsXNet functionality.

### <sup>403</sup> 2.3.3 Evaluation

<sup>404</sup> Although the utility of the proposed brain parcellation framework is highly dependent on  
<sup>405</sup> the specific application, we demonstrate the utility through the generation of cortical thick-  
<sup>406</sup> ness maps<sup>86</sup> which leverages both brain parcellation and the capabilities of mouse brain-  
<sup>407</sup> based isotropic interpolation for anisotropic data. Cortical thickness has demonstrated util-  
<sup>408</sup> ity in both human (e.g.,<sup>85,87</sup>) and non-human data (e.g., canines<sup>88</sup>, dolphins<sup>89</sup>, non-human

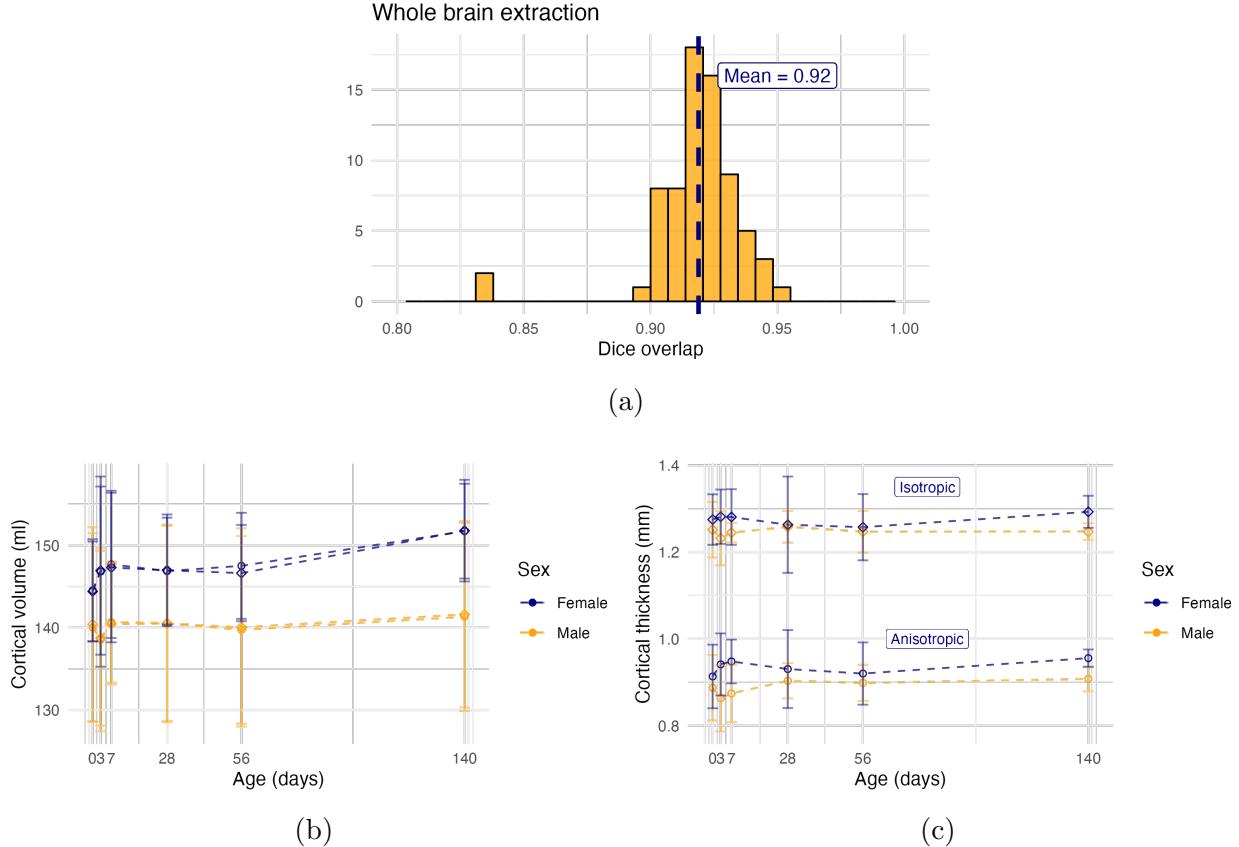


Figure 8: Evaluation of the ANTsX mouse brain extraction, parcellation, and cortical thickness pipeline on an independent dataset consisting of 12 specimens  $\times$  7 time points = 84 total images. (a) Dice overlap comparisons with the user-generated brain masks provide good agreement with the automated results from 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) The volumetric comparative results contrast with the cortical thickness measurements which illustrate estimation in anisotropic space severely underestimates the actual values in comparison with the isotropic prediction.

<sup>409</sup> primates<sup>90</sup>) including the mouse brain<sup>41,91–93</sup>.

<sup>410</sup> For evaluation, we used an additional publicly available dataset<sup>70</sup> which is completely inde-  
<sup>411</sup> pendent from the data used in training the brain extraction and parcellation networks. Data  
<sup>412</sup> includes 12 specimens each imaged at seven time points (Day 0, Day 3, Week 1, Week 4, Week  
<sup>413</sup> 8, Week 20) with in-house-generated brain masks. Spacing is anisotropic with an in-plane  
<sup>414</sup> resolution of  $0.1 \times 0.1 mm^2$  and a slice thickness of  $0.5 mm$ . Since the training data is isotropic  
<sup>415</sup> and data augmentation includes downsampling in the canonical directions, each of the two  
<sup>416</sup> networks learns mouse brain-specific interpolation such that one can perform prediction on  
<sup>417</sup> thick-sliced images, as, for example, in these evaluation data, and return isotropic probabil-  
<sup>418</sup> ity and thickness maps (a choice available to the user). Figure 8 summarizes the results of  
<sup>419</sup> the evaluation and comparison between isotropic and anisotropic cortical measurements in  
<sup>420</sup> male and female specimens.

421 **3 Discussion**

422 The diverse mouse brain cell type profiles gathered through BICCN and associated efforts  
423 provides a rich multi-modal resource to the research community. However, despite significant  
424 progress, optimized leveraging of these valuable resources is ongoing. A central component  
425 to data integration is accurately mapping novel cell type data to into CCFs for subsequent  
426 processing and analysis. To meet these needs, tools for mapping mouse cell type data must  
427 be both generally accessible to a wide audience of investigators, and capable of handling  
428 distinct challenges unique to each data type. In this work, we described modular ANTsX-  
429 based pipelines developed to address the needs of three BICCN projects that cover distinct  
430 cell type data, including spatial transcriptomic, morphological, and developmental data. We  
431 highlighted how a modular toolbox like ANTsX can be tailored to address problems unique  
432 to each modality through leveraging a variety of ready-to-use powerful tools that have been  
433 previously validated in multiple application scenarios.

434 Our MERFISH pipeline provides an example of how to map high-resolution spatial transcripto-  
435 mic data into the AllenCCFv3. While the techniques employed for mapping the sectioned  
436 data can be generally applicable to spatially transform other serial histology images, much  
437 of the pipeline was designed to specifically address known alignment challenges in the MER-  
438 FISH data. Thus pipeline shows how general ANTsX tools can be adapted to target highly  
439 specialized problems in mouse cell type data.

440 In contrast to the MERFISH pipeline, our fMOST pipeline was designed to be a more general  
441 solution that can be employed in other modalities. The pipeline primarily uses previously  
442 developed ANTsX preprocessing and atlasing tools to map fMOST data into the AllenCCFv3.  
443 The key component of the pipeline is the use of a fMOST-specific average shape and intensity  
444 atlas to most effectively address image registration in this context. The mapping between  
445 the fMOST atlas is generated once and reused for each new fMOST image. Lastly, ANTsX  
446 provides point set transformation tools to allow the mappings found through the pipeline to  
447 be directly applied to associated single-cell reconstructions from the fMOST data to study  
448 neuronal morphology.

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

463 We also presented a mouse brain morphological pipeline for brain extraction and brain  
464 parcellation 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 anticipate that refinements will be necessary. Given that the ANTsX  
468 toolkit is a dynamic effort undergoing continual improvement, we manually correct cases that  
469 fail and use them for future training and refinement of network weights as we have done for  
470 our human-based networks. Generally, these approaches provide a way to bootstrap training  
471 data for manual refinement and future generation of more accurate deep learning networks  
472 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. The  
476 extensive functionality of ANTsX makes it possible to create complete processing pipelines  
477 without requiring the integration of multiple packages or lengthy software development.

<sup>478</sup> These open-source components not only perform well but are available across multiple plat-  
<sup>479</sup> forms which facilitates the construction of tailored pipelines for individual study solutions.  
<sup>480</sup> These components are also supported by years of development not only by the ANTsX  
<sup>481</sup> 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 algorithm<sup>52</sup> 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>
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 technique<sup>61</sup> 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 scenarios<sup>94</sup>. It includes innovative transformation models  
512 for biological modeling<sup>51,67</sup> and has proven capable of excellent performance<sup>56,95</sup>. Vari-  
513 ous parameter sets targeting specific applications have been packaged with the different  
514 ANTsX packages, specifically ANTs, ANTsPy, and ANTsR<sup>46</sup>. 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 aggressive alternative. Transforming images using the derived transforms is per-  
520 formed 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 Gaussian<sup>51</sup> or B-spline regularization<sup>67</sup> 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 described<sup>59</sup> and recently used to create the DevCCF templates<sup>16</sup>.

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) algorithm<sup>51</sup>. 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 correction<sup>52</sup>, `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       functionality<sup>94</sup> 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 projections<sup>79</sup>. 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 technique<sup>84</sup>, 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 templates<sup>16</sup>, 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<sup>46,96</sup>. 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 architecture<sup>97</sup> 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 data<sup>69</sup> which were combined to synthesize isotropic volu-  
742 metric data using the B-spline fitting algorithm<sup>84</sup>. 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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## <sup>774</sup> Author contributions

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

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