Identifying partial mouse brain microscopy images from Allen reference atlas using a contrastively learned semantic space

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Published in: arXiv

Publication date: 2021

IDENTIFYING PARTIAL MOUSE BRAIN MICROSCOPY IMAGES FROM ALLEN REFERENCE ATLAS USING A CONTRASTIVELY LEARNED SEMANTIC SPACE

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ABSTRACT

Precise identification of mouse brain microscopy images is a crucial first step when anatomical structures in the mouse brain are to be registered to a reference atlas. Practitioners usually rely on manual comparison of images or tools that assume the presence of complete images. This work explores Siamese Networks as the method for finding corresponding 2D reference atlas plates for given partial 2D mouse brain images. Siamese networks are a class of convolutional neural networks (CNNs) that use weight-shared paths to obtain low dimensional embeddings of pairs of input images. The correspondence between the partial mouse brain image and reference atlas plate is determined based on the distance between low dimensional embeddings of brain slices and atlas plates that are obtained from Siamese networks using contrastive learning. Experiments showed that Siamese CNNs can precisely identify brain slices using the Allen mouse brain atlas when training and testing images come from the same source. They achieved TOP-1 and TOP-5 accuracy of 25% and 100%, respectively, taking only 7.2 seconds to identify 29 images.

Index Terms—Mouse brain, Partial data, Contrastive learning

1. INTRODUCTION

Determining the location of anatomical structures in a mouse brain is an essential step for analyzing and understanding the architecture and function of brain circuits and of the overall whole-brain activity [1]. Anatomical structures can be located using standardized anatomical reference atlases, usually taking a two-step approach:

1. Identification: The input brain slice has to be identified, i.e., the corresponding 2D atlas plate has to be found.
2. Registration: The identified slice is registered to the corresponding atlas plate. Anatomical structures are determined based on the registered annotated plate.

However, the acquired images of brain slices often suffer from artifacts due to missing tissue parts, irregular staining, air bubbles and tissue wrinkles [2]. This is further aggravated due to variations in the images, depending on the experimental procedures, instrumentation noise, etc. This makes it difficult to identify and register mouse brain images. For this reason, practitioners usually resort to manually comparing image slices to 2D atlas plates which can be very time-consuming.

In standard image registration methods, the moving image $I_M$ is spatially aligned to the fixed image $I_F$ by applying a coordinate transformation to the moving image, $T(\cdot) : I_M \mapsto I_F$. The optimal transformation $T(\cdot)$ is obtained by minimizing the cost function $C(T(\cdot), I_F, I_M)$ [3]. The downside of conventional image registration methods is that the cost function for each registration task is optimised from scratch limiting the registration accuracy and speed. Many recent studies have tried to overcome this problem by using artificial neural networks [4] [5] [6].

Compared to the registration of mouse brain images, the first part of identification has received far less attention from the research community. However, wrong identification of brain slices would lead to incorrect determination of anatomical structures regardless of how well image registration is performed. Therefore, precise determination of anatomical structures at first requires precise identification of brain slices.

The correspondence between brain slices and atlas plates can be found by reconstructing a 3D volume from the brain slices and then registering it to the 3D reference atlas [7]. However, it is not always possible to construct an accurate brain volume, e.g. when brain slices are cut at different angles, only several brain slices are available or partial brain images are used.

This study investigates the problem of identifying partial 2D mouse brain slices. The missing data could either be due to acquisition artifacts or cases where only portions of the brain regions are of interest. Brain slices are identified by finding corresponding 2D coronal plates in the Allen Mouse Brain Atlas [8]. This is achieved by using Siamese Networks. Siamese CNNs are trained to learn low dimensional representations (embeddings) of images. Then the correspondence between brain slices and atlas plates is determined based on the distance between these embeddings. CNNs are implemented, trained and compared with a baseline method based on conventional image registration. The proposed method is compared in terms of accuracy and speed to SimpleElastix which
Fig. 1. Computing the similarity between brain slices and atlas plates with Siamese Networks. Low dimensional representations corresponding to the moving and fixed images are obtained as output from the identical CNNs which are further used to compute their pairwise similarity.

is based on the widely used tool Elastix [9].

Once brain slices are identified, they can be affinely registered to the corresponding atlas plates using CNNs (Appendix B).

2. METHODS

Siamese networks are a class of artificial neural network architectures consisting of two or more sister (i.e. identical) networks. They can be used to find the similarity between images even when limited training data is available, e.g. in one one-shot learning tasks [10].

In this work, Siamese Networks are used to identify brain slices comprising two identical CNNs, $S_\theta(\cdot)$, where $\theta$ are the shared network parameters. At first, feature vectors (embeddings) for brain slices treated as the fixed image are obtained as $h_F = S_\theta(I_F) \in \mathbb{R}^L$, where $L$ is the dimensionality of the low dimensional embedding. The embeddings for the atlas plates treated as the moving image are obtained in a similar manner, $h_M = S_\theta(I_M) \in \mathbb{R}^L$. Then the similarity between brain slices and atlas plates is determined based on the Euclidean distance between their feature vectors, $d(h_M, h_F)$. The distance between images in this low dimensional embedding space is inversely proportional to their similarity i.e., the distance between the embeddings of more similar images is smaller than for dissimilar images. The reference atlas plate with the lowest Euclidean distance is then considered as the corresponding plate for a given brain slice.

The Siamese networks for brain slice identification are trained to learn the representation of images such that corresponding brain slices and atlas plates would be closer to each other in the embedding space. The networks are trained with two different loss functions:


$$L = \begin{cases} 
\frac{1}{2}d(h_F, h_M)^2 & \text{if positive pair} \\
\frac{1}{2} \max(0, m - d(h_F, h_M))^2 & \text{if negative pair} 
\end{cases} (1)$$

where positive pair means that two images belong to the same class and negative pair that two images belong to different classes.

2. Triplet loss [12]

$$L = \max(d(h_A, h_P) - d(h_A, h_N) + m, 0) (2)$$

where $h_A$, $h_P$, $h_N$ - the embeddings of anchor $I_A$, positive $I_P$ and negative $I_N$ images, respectively, and $m \in \mathbb{R}_+$ is the margin.

Note that in case of triplet loss, a third sister network with shared weights is included to obtain feature embeddings. An overview of the use of Siamese networks for atlas plate prediction with moving and fixed images is shown in Fig. 1.

Two different types of triplets $(I_A, I_P, I_N)$ are sampled to calculate the triplet loss. These triplets are defined based on the distance between the embeddings $h_A$, $h_P$, $h_N$ of anchor $I_A$, positive $I_P$ and negative $I_N$ images:

1. Semi-hard triplets: the distance between $h_A$ and $h_P$ is smaller than the distance between $h_A$ and $h_N$, however, the loss is still positive.

2. Hard triplets: the distance between $h_A$ and $h_N$ is smaller than the distance between $h_A$ and $h_P$.

Intuitively, when the models are trained with contrastive- and triplet- losses, embeddings of similar images are pulled together while embeddings of dissimilar images are pushed away from each other. In this way, the networks can learn representations of different classes.

3. DATA & EXPERIMENTS

3.1. Data

Eighty-four high-resolution microscopy images of mouse brain slices were acquired at Kiehn Lab[2]. The size of the images varied between 17408 $\times$ 10240 and 25600 $\times$ 20480 pixels. Most of the images were partial as they were not capturing the entire brain slice. For instance, the cortex or the cerebellar cortex were captured partially or, in some images, were not captured at all as seen in first column of Fig. 2. The images of brain slices were preprocessed, cropped and equalized using Contrast Limited Adaptive Histogram Equalization (CLAHE) to reduce some artifacts. The dataset was split into four sets: training (50 images), validation 1 (12 images), validation 2 (10 images) and test (12 images).

The Allen Mouse Brain Atlas [8] was used as the reference atlas. It consisted of 132 Nissl-stained coronal plates spaced at 100 $\mu$m, seen in the second column of Fig. 2.

[2]https://in.ku.dk/research/kiehn-lab/
Fig. 2. Examples of incorrectly predicted atlas plates by the Siamese Networks. Columns: (1) brain slices from the test dataset; (2) ground truth atlas plates; (3) predicted atlas plates. The number in parentheses shows the label of the atlas plate, i.e. the position of the atlas plate in the reference atlas.

To increase the number of training samples for the Siamese Networks, data augmentation (affine transformation, cropping and padding, pepper noise) was applied to the training dataset - all 50 brain slices and all 132 atlas plates. The images were resized to $1024 \times 1024$, $512 \times 512$, or $224 \times 224$ depending on the experiment.

3.2. Experiments
The performance of the Siamese Networks was compared with a SimpleElastix-based algorithm that identifies brain slices based on mutual information (MI). The algorithm affinely registers each brain slice with every atlas plate and picks the atlas plate with the highest MI. In total, 100 random hyperparameters from the SimpleElastix affine parameter map were tested (Appendix A). The results of the best performing baseline model are reported.

Metrics: The methods were evaluated based on three metrics: Mean Absolute Error (MAE), TOP-N accuracy and inference time. MAE measured the accuracy of predictions. For each brain slice all 132 atlas plates were ranked (starting from zero) based on the similarity score (the Euclidean distance or MI, depending on the method). Then MAE was computed as

$$MAE = \frac{\sum_{i=0}^{N} |\tilde{y}_i|}{N},$$

where $N$ is the number of brain slices, $\tilde{y}_i$ is the position of ranked ground truth atlas plate for a given brain slice $i$. With 132 atlas plates used, MAE can have values in the range $[0, 131]$. If all brain slices are identified correctly, MAE is equal to 0.

Hyperparameters: Fig. 3 shows the architecture of the Siamese Networks with the embedding space feature dimension $L = 64$. The base of the Siamese Networks consists of a ResNet network [13] pre-trained on the ImageNet dataset. While training the Siamese Networks, all layers of the ResNets were frozen except the last ones starting with the prefix conv5. The networks were trained on the training dataset for a maximum of 10,000 iterations using the Adam optimizer [14] with an initial learning rate of $10^{-4}$. The experiments were conducted using Nvidia GeForce RTX 3090 GPU, i7-10700F CPU and 32 GB memory. The training was stopped if MAE on the validation 1 dataset was not decreasing for more than 2,000 iterations. Later, the models were evaluated on the validation 2 dataset (Table 1). The best performing model was compared with the SimpleElastix-based approach on the test dataset (Table 2). Table 3 shows the predicted atlas plates on the subset of the test dataset. Examples of the predicted atlas plates by the Siamese Networks are presented in Fig. 2.

4. DISCUSSIONS & CONCLUSIONS
The Siamese networks used to identify brain slices have shown impressive results, i.e. in finding corresponding coronal 2D atlas plates. It achieved TOP-5 accuracy of 100% meaning that the actual corresponding atlas plate always falls in the top 5 predicted atlas plates. The identification accuracy (MAE) had no clear correlation with the batch size (16 and 32), the image resolution ($224 \times 224$, $448 \times 448$, $1024 \times 1024$) and the type of the base for the Siamese network (ResNet50v2 and ResNet101v2). However, using images with lower resolution and networks with fewer parameters could improve the inference time. We did not observe that the performance of the Siamese network would be highly influenced by the loss function, namely contrastive and triplet losses. The models trained with triplet loss rather than contrastive loss, on average, achieved higher accuracy, however, the difference is not significant.

The Siamese networks produced impressive results even though some images of different classes looked very similar to each other, thus making the identification task even more complex. The distance between such images should be lower than the distance between two completely dissim-
Table 1. Mean Absolute Error (MAE) on the validation dataset for identifying brain slices with the Siamese Network. The lowest MAE is achieved by the network with ResNet50v2 base, trained with semi-hard triplet loss and using 1024 × 1024 images.

<table>
<thead>
<tr>
<th>Loss</th>
<th>Batch size</th>
<th>224x224</th>
<th>448x448</th>
<th>1024x1024</th>
<th>224x224</th>
<th>448x448</th>
<th>1024x1024</th>
</tr>
</thead>
<tbody>
<tr>
<td>Triplet (semi-hard)</td>
<td>32</td>
<td>2.5</td>
<td>2.2</td>
<td>2.8</td>
<td>1.9</td>
<td>3.1</td>
<td>3.1</td>
</tr>
<tr>
<td></td>
<td>16</td>
<td>2.0</td>
<td>3.7</td>
<td><strong>1.8</strong></td>
<td>2.6</td>
<td>2.1</td>
<td>2.7</td>
</tr>
<tr>
<td>Triplet (hard)</td>
<td>32</td>
<td>2.4</td>
<td>3.0</td>
<td>3.0</td>
<td>2.8</td>
<td>3.7</td>
<td>2.7</td>
</tr>
<tr>
<td></td>
<td>16</td>
<td>3.1</td>
<td>2.8</td>
<td>2.6</td>
<td>2.0</td>
<td>2.7</td>
<td>2.8</td>
</tr>
<tr>
<td>Contrastive</td>
<td>32</td>
<td>3.6</td>
<td>2.1</td>
<td>3.4</td>
<td>4.2</td>
<td>2.5</td>
<td>5.6</td>
</tr>
</tbody>
</table>

Table 2. Performance of CNN-based and the SimpleElastix-based approaches on the test dataset for identifying brain slices. Siamese network outperforms SimpleElastix-based approach by a large margin in all evaluated metrics. Inference time measures how long it takes to identify all 12 brain slices from the test dataset.

<table>
<thead>
<tr>
<th></th>
<th>MAE</th>
<th>TOP-1</th>
<th>TOP-3 Acc</th>
<th>TOP-5 Acc</th>
<th>TOP-10 Acc</th>
<th>Inference time</th>
</tr>
</thead>
<tbody>
<tr>
<td>SimpleElastix</td>
<td>60.4</td>
<td>16.7%</td>
<td>25%</td>
<td>25%</td>
<td>25%</td>
<td>12 hours 25 mins</td>
</tr>
<tr>
<td>Siamese Networks</td>
<td><strong>1.42</strong></td>
<td><strong>25%</strong></td>
<td><strong>83.3%</strong></td>
<td><strong>100%</strong></td>
<td><strong>100%</strong></td>
<td><strong>7.2 sec</strong></td>
</tr>
</tbody>
</table>

Table 3. Identifying brain slices from the subset of the test dataset: the labels of ground truth and top-5 predicted atlas plates by the Siamese Networks. Even though some predictions are incorrect, all of them are close to the ground truth labels. Labels define the position of atlas plates in the reference atlas.

<table>
<thead>
<tr>
<th>Ground truth atlas plate</th>
<th>Top 5 predicted atlas plates (sorted by similarity score)</th>
</tr>
</thead>
<tbody>
<tr>
<td>91</td>
<td>92, 91, 93, 90, 94</td>
</tr>
<tr>
<td>130</td>
<td>129, 128, 130, 131, 127</td>
</tr>
<tr>
<td>86</td>
<td>87, 88, 86, 85, 89</td>
</tr>
<tr>
<td>63</td>
<td>62, 61, 60, 63, 59</td>
</tr>
<tr>
<td>108</td>
<td>109, 110, 111, 112, 108</td>
</tr>
</tbody>
</table>

ilar images. Maximizing the distance between all images of different classes would make it difficult for networks to learn representations of these classes. Contrastive and triplet losses solve this issue by using margin, i.e., dissimilar images are not pushed away if the distance between them is larger than the margin.

In this study, we proposed Siamese Networks as a method for identifying correspondence between complete and partial mouse brain slices, i.e. finding the corresponding 2D atlas plates. The networks have shown a high precision and significantly improved inference time compared to the baseline. While we demonstrated this with a 2D reference atlas, the same method can also be applied to a 3D reference atlas for even higher identification precision.

Acknowledgments The authors would like to thank Kiehn Lab (University of Copenhagen, Denmark) for providing access to the microscopy images and the hardware used to train the models. They also acknowledge the Core Facility for Integrated Microscopy (CFIM) at the Faculty of Health and Medical Sciences for support with image acquisition.

5. REFERENCES


A. SIMPLEELASTIX HYPERPARAMETERS

A.1. Hyperparameters in the random search:

- Automatic parameter estimation: true, false
- Automatic scales estimation: true, false
- Number of resolutions: 1, 2, 3, 4, 5, 6, 7
- Maximum number of iterations: 200, 400, . . . , 3000
- Use random sample region: true, false

• Fixed and moving image pyramid: Recursive ImagePyramid, ShrinkingImagePyramid, SmoothingImagePyramid

A.2. Fixed parameters

- NumberOfSpatialSamples: 10000
- NumberOfSamplesForExactGradient: 100000
- NumberOfSamplesForExactGradient: 64
- Metric: MutualInformation
- Interpolator: Linear
- Optimizer: AdaptiveStochasticGradientDescent

B. AFFINE REGISTRATION USING CNNS

After finding candidate atlas plates, the brain images can be registered to the atlas plates. In this section, a CNN-based affine registration network is proposed, evaluated and compared to the conventional image registration tool SimpleElastix.

B.1. Methods

The affine transformation matrix is given by

\[ T = \begin{pmatrix} a_{11} & a_{12} & t_x \\ a_{21} & a_{22} & t_y \end{pmatrix}, \]

which is applied to each pixel in the moving image \( I_M \). The affine registration parameters are learned using the regression network, \( R_\phi(\cdot) \), implemented using a CNN with parameters \( \phi \). The regression network is trained in two stages to predict the six affine parameters in (3). In the first step, a form of pre-training is performed using only the atlas plate images. Atlas plate images are transformed using random transformations and the network is trained to predict these affine parameters. In the second step, the weights of the network are fine tuned by registering brain slices with atlas plates. The fine tuning is performed in the same way as the training in the first stage, except that the inputs now consist of a brain slice and its corresponding atlas plate. In both stages, mutual information is used as an objective function. An overview of the regression network used to predict the affine transformation parameters is shown in Fig. 4.

B.2. Data

Data consisted of twenty-seven partial brain slices and coronal plates from the Allen Mouse Brain Atlas (CCFv3). Only a subset of atlas plates was used in the experiments, i.e. 132 Nissl-stained and 132 average template images.
Fig. 4. Training the regression network to predict random affine transformations applied to atlas plates. In the pre-training step, two corresponding atlas plates are taken with one of them is used as a moving image, and the other one as a fixed image after applying a random affine transformation. The fixed image is slightly cropped to resemble a partial brain slice, concatenated with the moving image and passed through the network. The output of the network is a 2 × 3 affine transformation matrix which is applied to the moving image. The mutual information is calculated between the moved and fixed images. In the second stage, for fine tuning the brain slice and the atlas plate identified using Siamese networks are used in the same set-up without any additional random transformations.

B.3. Experiments

The regression network for affine registration consisted of 7 convolutional blocks (convolutional 2D layer + max pooling) and 2 fully connected layers with 256 and 6 neurons. The final 6 parameters represented a 2×3 affine transformation matrix. To ensure that all affine parameters are in a similar range, the translation parameter was measured as a fraction of the image height/width, e.g. 0.5 denoted half of the axis size. In the first stage, the regression network was trained for 3000 iterations and in the second stage for 100 iterations using the Adam optimizer with an initial learning rate of $10^{-4}$.

SimpleElastix served as a baseline for the affine registration task. The registration was performed using the default affine parameter map. Only the maximum number of iterations was changed to 3000 and the number of voxels sampled in each iteration to compute the cost function to 100,000.

Registered brain slices had no ground truth affine parameters or structure masks, therefore, the registration accuracy was determined based on a qualitative analysis, i.e. visual comparison of registered images (Fig. 5). Based on the qualitative analysis from all 27 brain slices, the regression network achieved much higher registration precision than SimpleElastix.

The regression network was not only more accurate but also considerably faster than the baseline. It took 179 seconds to register one brain slice with SimpleElastix while the inference time with CNNs was less than a second. However, it took additional 70 seconds on the CPU (14 seconds on the GPU) to train the networks on a specific image registration task for 100 iterations.

B.4. Discussion

CNNs have been shown to be more accurate than SimpleElastix in affine image registration when partial data is used. However, not all images were registered precisely (e.g. row (c) in Fig. 5). Local MI might be a better option than global MI when images have uneven brightness, as it was the case with the brain slices. Therefore, the results might be improved by using local MI as an objective function.
Fig. 5. Affine registration on the subset of the test dataset. Average template images from the Allen Mouse Brain Atlas (second column) are registered to the brain slices (first column) using CNNs (third and fourth column) and SimpleElastix (fifth column).