

# Robust Landmark Detection for Alignment of Mouse Brain Section Images

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**Abstract.** Brightfield and fluorescent imaging of whole brain sections is a fundamental tool in anatomical studies on brain functions. As automation of the sectioning and image acquisition processes become more efficient, there is an increasing need to automate the post-processing of sections for alignment and three dimensional visualization. There is a further need to facilitate the development of a “digital atlas”, i.e. a brain-wide map annotated with cell type, tract tracing and physiological data, which serves as the underpinning of circuits analysis as well as for quantitative analysis of phenotypical variation. Currently, such maps are created by hand.

In this work we describe the first steps in developing a semi-automated system to construct a mouse brain atlas that combines annotation, registration and atlas building in an iterative framework. This atlas stores landmark models, uses them to detect landmarks from new images and maps the images onto the atlas’s standardized coordinate system; meanwhile, the models in the atlas are updated based on newly registered images.

The aspect of the project described here is a semi-supervised learning system for identifying landmarks. It detects and models regions with distinct texture that are consistent across sections. It also identifies boundary segments for regions that are not compact. Experiments show that the detected landmarks correspond well with labellings by experienced anatomists. The results serve as a promising basis for the next stage of registration and atlas building.

**Keywords:** landmark detection, atlas building, mouse brain, registration, automated annotation

## 1 Introduction

Brightfield and fluorescent imaging is of central importance in mouse brain studies. As automation is improving, a whole brain can be imaged in a matter of hours. At the same time, there is an increasing number of fluorescent markers that can be used to label neurons with specific phenotypes. As the number of available images mounts, the manual work required to analyze these images becomes a major bottleneck and tools that aggregate information in the images such as standardized atlases become necessary. A standardized atlas maps image

series of different specimens onto a common 3D coordinate system. On the one hand, this allows the new specimen to be compared with annotations and cell type maps collected from previous specimens; on the other hand, new specimens augment the atlas by introducing annotations for new structures or by providing additional cell type data demonstrated by new markers.

Much effort has been made to construct digital atlases for a variety of species. A popular atlas for mice is the Allen Reference Atlas. It consists of section images of a single brain, with major anatomical structures annotated and localized in the 3D reference space. While this atlas is useful as a visual guide for annotating new images by hand, there is no easy solution for aligning new image series to the atlas. Software for registration usually requires heavy human intervention, such as picking the landmarks or adjusting parameters that lack intuitive meaning.

We propose building an “active” atlas. In such an atlas, characteristics of annotated regions are modelled using machine learning. These models are then used to detect similar regions in new images. Detected regions serve as landmarks for both intra-specimen registration and specimen-atlas registration. Once the new images are aligned to the atlas, region models are updated based on characteristics of detected regions in the aligned new images. Essentially, the atlas “actively” adapts to registered specimens, and “actively” annotates new specimens for easier registration. In contrast to most existing work that either focuses on building an atlas by registering a single image series, or on aligning image series to an fixed atlas, the active atlas combines annotation, registration and atlas building into an iterative framework that is particularly suitable for processing a large number of specimens in an incremental fashion.

One crucial component in building an active atlas is identification of the landmarks. Until the region models are trained with sufficiently many examples, the system must obtain the annotation in other ways. Having human labellers do the annotation is time-consuming. In this paper, we propose an automatic approach.

Our particular study focuses on the brainstem. Unlike the cerebrum and the cerebellum, where neurons form high-contrast layered structures such as the cortex, the brainstem mostly contains nuclei, which are compact clusters of neurons. For example, Figure 1 shows the facial motor nucleus. Neurons in each nucleus have similar size, shape and stain sensitivity, often also demonstrating uniform density and particular directionality. This gives each nuclei a distinctive texture, allowing them to be robustly identified when building the atlas. The fact that they are well localized also makes them perfect landmarks for registration. In addition to nuclei, fiber tracts are also recognizable structures in the brainstem. In particular, the directionality of a tract serves as an important feature of the landmark.

We therefore aim to model the landmarks based on texture. Section 3 describes how we represent textures using Gabor filters and superpixels. Section 4 explains how we identify potential landmark regions based on texture distinctiveness. Section 6 describes how we detect boundary segments based on regions

in order to improve robustness. Section 7 describes how landmarks are matched between sections.

## 2 Related Work

Intensity based Registration Mostly intensity based

Point Landmark Detection

SIFT

Saliency and Objectness Detection

global rarity scheme

center-surround scheme

[\[YF: Is there work that uses notion of statistical significance in this context?\]](#)

Texture Representation

gabor filter

textons

## 3 Representing Texture using Histograms of Gabor Textons

Images in the stack are filtered using Gabor filters with nine orientations and ten scales. From each image, 1000 feature vectors are sampled. We reduce the data by quantizing these 99 dimensional feature vectors using K-Means. First, 100 clusters are generated; then clusters with close centroids are merged. The final cluster centroids are the textons.

We further reduce the data by describing texture at the level of superpixels. For each superpixel, texture is represented by the histogram of the textons it contains. The superpixels are obtained using the SLIC algorithm.

Since different sections may be oriented at different angles, and in order for two identical but slightly rotated textures to match, we need the features to be rotation-invariant. To this end, we compute the energy distribution over orientations for each feature vector, and shift all feature vectors such that the modes of directional energy distributions are aligned. In this way, the texture is decoupled from directionality. Note that the directionality is still useful, particularly for describing fiber tracts.

## 4 Evaluating Region Significance using Statistical Test

There are three factors in determining the significance score of a region. The first factor is the distinctiveness of this region's texture, or in other words, how different its texture is from the surrounds. Since textures are represented as histograms, this problem naturally leads to a statistical test on whether the region's average texton histogram and those of its surrounds are sampled from the same distribution. In our experiment, the chi-squared test is used. The p-value of this test can be used as a quantitative measure of the distinctiveness. Since a region

often has many neighbouring regions with different textures, the test is made between the region’s histogram and that of every surrounding superpixel, and the largest p-value is used.

The second factor is the texture homogeneity within the region. This is defined in terms of the mean dissimilarity between all superpixels in the region and the region average.

The third factor stems from the fact that most nuclei have compact shapes. A common measure for the compactness of a closed curve is the isoperimetric quotient, defined as the square of the circumference divided by the enclosing area. We use the number of superpixels in the region as a proxy for the area and the number of surrounding superpixels as that for the circumference.

## 5 Finding Significant Regions with Region Growing and Clustering

Now that textures are represented using histograms of Gabor textons, we need to find regions with distinct texture. In this section, we describe a method for finding potential regions as sets of connected superpixels. We also propose a score that measures the significance of a region.

Region growing is performed on every superpixel. Starting from a singleton containing the seed superpixel, the greedy procedure considers all neighbours of the current set, and iteratively adds the one whose texton histogram is closest to the current region histogram. The growing stops when the region reaches 10% of the total area. Eventually, the region at the point when the significance score is the largest is returned. Figure x shows examples of this procedure.

We call the set of superpixels that grows from a seed the *expansion region* of the seed. If a region has distinct texture, the expansion regions grown from the superpixels inside this region are expected to be almost identical, and they will have no overlap with the expansion regions from superpixels outside of this region (see Figure x). In other words, the expansion regions form clusters. The denser a cluster is, the more significant the region it represents. Using Jaccard index as the pairwise distance, we group the expansion regions using hierarchical clustering. We then find the groups whose size is large enough, and select the expansion region with the highest significance score as the representative of that group.

Finally, the representative expansion regions from all groups are ranked according to the significance scores. Figure x shows the top 10 regions.

How to model the regions...

## 6 Evaluating Boundary Robustness by Region Consensus

In addition to compact-shaped regions, boundaries between structures are also useful landmarks. In many cases, nuclei are separate from each other and have clear closed contours. In other cases, a region has a clear boundary on one side,

but on the other side is a gradual transition into the neighboring texture. One example is shown in Figure x. For these regions only partial boundary can be identified.

As region growing is sensitive to the seed, the expansion regions from different seeds in the same anatomical structure are not always consistent. However, most of them will agree on parts of the boundary that are stable (see Figure x). This motivates a region consensus approach for identifying the robust boundary segments.

The strategy is to let each expansion region vote for every of its boundary segment. The weight of the vote cast to a particular segment depends on the chi-squared distance between the region average and the superpixel at the other side of the segment. The boundariness score for a segment  $(i, j)$  is:

$$b((i, j)) = \sum_{i:j \in \delta S_i} \frac{d(\bar{h}_i, h_j)}{|S_i|}$$

We also record the set of superpixels that vote for a segment, and call it the *supporter set* of the segment.

We discard boundary segments whose vote is lower than a threshold and model the remaining segments. Instead of modelling individual segments separately, we want to group segments that receive votes from the same group of expansion regions. Therefore we define the association between two boundary segments to be the Jaccard index of their supporter sets, and apply hierarchical clustering to obtain boundary segment groups. Each group represents a partial boundary. All partial boundaries are ranked according to the total vote of its segments.

## 7 Matching Boundaries from Different Sections

In this section, we design a distance function for comparing two partial boundaries. This function is a weighted combination of the differences in four aspects.

The first term is the difference in two interior textures. This is simply the  $\chi^2$  distance between the texton histograms.

The minimum bipartite matching for the two sets of boundary segments is computed using the Hungarian algorithm.

The shape context descriptors characterize the local shape around each of a set of sample points using a histogram.

The distance between two segments depends both on the similarity of the shape context descriptors and on the similarity of the texture at the exterior of the boundary segment.

For each boundary, the shape context descriptors are computed for the point set consisting of the midpoint of every boundary segment.

the exterior of the boundary segment

The fourth term is the spatial distance between the boundaries. This is the Euclidean distance between the center of mass of the boundaries' midpoint sets.

$$D(c_1, c_2) = w_{int} \cdot \chi^2(\bar{h}_1, \bar{h}_2) + w_{ext} \cdot \max_{i \in \delta c_1, j \in \delta c_2} (h_i, h_j) + w_{sha} \cdot \sum_{i \in \delta c_1, j \in \delta c_2, i, j \text{ matches}} \chi^2(s_i, s_j)$$

Boundaries are detected from two sections and their pairwise distances are computed. A pair of boundaries are matched if they are both the closest boundary of one another.

## 8 Experiments

### 8.1 comparison with human labelings

We test our algorithm on a series of section images of a rat brainstem, stained with Cresyl Violet for Nissl substance in neurons. The images are scanned at 2 microns per pixel, showing individual neuronal cell bodies. Two experienced anatomists are asked to label the same images for anatomical regions that are recognizable from the images .

### 8.2 robustness of matching

Shows that matchings are robust to distortion and shape change. Also shows that our distance measure is a sensible one: each of the four terms is important. We show this by changing the term weightings, and then compare matching results.

## 9 Future Work

use detected landmarks for registration

Learn dictionary using deep neural networks, ICA, ...

## References

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