TOWARD AUTOMATED REGION DETECTION & PARCELLATION

OF RAT BRAIN TISSUE IMAGES

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TOWARD AUTOMATED REGION DETECTION & PARCELLATION OF RAT BRAIN TISSUE IMAGES

by

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THESIS

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Abstract

People who analyze images of biological tissue often rely on segmentation of structures as a preliminary step. In particular, laboratories studying the rat brain manually delineate brain regions to position scientific findings on a brain atlas to propose hypotheses about the rat brain, and ultimately, the human brain. Our work intersects with the preliminary step of delineating regions in images of brain tissue via computational methods.

We investigate pixel-wise classification or segmentation of brain regions using ten histological images of brain tissue sections stained for Nissl substance, and two deep learning models: U-Net and Tile2Vec. Our goal is to assess the viability of segmenting brain structures from images alone with both supervised and unsupervised approaches. Further, we determine how image resolution and additional domain knowledge affects segmentation.

Experimenting with different resolutions shows the supervised model performs best when the data has enough resolution to distinguish cytoarchitectural patterns. At the same time additional domain knowledge, in the form of atlas-guided parcellations, improves segmentation for some cases while misclassification occurs in other cases. We argue misclassification is partly due to limited availability of data, rendering the supervised model incapable of performing well on data it has never seen. To this end, we employed an unsupervised approach where the goal is to generate lower dimensional representations of image tiles taken from the histological images. This approach is data efficient and enables segmentation of different structures with traditional computer vision techniques. Overall, our work shows segmenting structures is possible with histological images of brain tissue sections stained for Nissl substance.

Our efforts contribute to a long history of characterizing the brain. We continue working with intentions of, one day, streamlining reconstruction of rat brain maps, and other animal models, using histological data.

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Chapter 1

Introduction

Our work investigates methods to automate the development of digital rat brain maps via computer vision and learning algorithms.

1.1 Setting

There is an encouraging development to map neuroscientific data under a standardized atlas framework [22] [29]. Positioning data, such as genetic information and injection deposits, on an agreed-upon map is argued to render an accessible and comparable representation of the original data [44]. Moreover, it ensures the legacy of the data and allows future researchers to reference previous work at the same location on the map. Legacy data could provide context and enrich new research, helping reveal trends across studies [22]. Additionally, atlases provide a means to validate experimental procedures, such as targeting regions of interest for injections or electrode placement [43] [39]. In sum, mapping to a standardized atlas is a valuable practice for neuroscientists, and is impossible without first parcellating experimental brain sections.

Several methods to parcel or segment brain regions exist and can be grouped under two umbrella categories: functional and structural. Functional parcellations can be broken down by definitions of function: parcellating regions theorized to be connected in a system, or that are engaged during certain activities. Similarly, structural parcellations can be broken down by definitions of structure: parcellating regions based on presence of myelin (gray and white matter) or cell population boundaries [15]. Computationally, segmentation based on function is possible with images generated by technologies like functional MRI,

while segmentation based on structure is possible with images generated by methods such as MRI, CT scans, or histological imaging. Segmenting rat brain regions tends to align with structural parcellation since most analysis is done with MRI, CT, or histological image data.

Importantly, the imaging method of choice will determine the degree of granularity in a segmentation. Presently, rat brain atlases are developed by considering cellular-level structures evident in high resolution images of appropriately stained brain sections [41]. However, despite the advances in tissue staining and microscopy, which refined the way neuroscientist develop rat brain maps, most computational techniques applied to segment brain regions are designed for human brain images. Typically, methods for human brain segmentation utilize data gathered by MRI and CT scans where images have a resolution comparable to or less than the resolution of the naked eye. As a result, the majority of segmentation algorithms designed to delineate brain regions do not consider cellular-scale features, or cytoarchitecture, present in histological images. Instead they consider similarity in pixel values or clear boundaries between objects of interest present in MRI and CT scans. Ideally, an algorithm designed to segment rat brain regions and sub-regions should make use of information found in high-resolution histology images and consider features beyond pixel value similarity or clear boundaries.

Consequently, deciding on the file format of the parcellation is equally important. Brain atlases typically take the form of a stack of 2D planes, or levels, to capture the organ's 3D nature. Each 2D plane is a map generated from a 2D image, and can take multiple formats ranging from paper to computer representations, although digital maps are the current standard. Vector graphics, for example, are a popular choice, since vector objects, such as lines and polygons, are easily modified in vector graphics software as independent elements. In fact, existing rat brain atlases are vector-based, and laboratories mapping to these atlases manually parcellate their experimental brain tissues with vector-based software. As an alternative, brain maps could be rendered in a raster format. Under a computer vision domain, a raster map is equivalent to assigning each pixel to exactly one

class or brain region. This alternative, however, is not preferred due to its rigidity in terms of scale and ability to interact with brain regions or mapped objects. Yet, despite its lack of popularity as a map format, computer algorithms that could potentially automate the generation of brain maps operate on raster data. To this end, we argue both vector and raster formats offer independent strengths for data integrity and portability, automation, and data analysis.

To conclude, brain maps are essential tools for clinicians and neuroscientists. These structural parcellations are often in digital form and are generated by different imaging modalities. In the case of rat brain maps, vector maps are generated by careful consideration of cytoarchitectural information in histology images. However, there is currently no segmentation techniques available that exploit cytoarchitecture to delineate brain regions.

1.2 Objective

Our goal is to develop segmentation techniques to automate human-level parcellation of brain regions. In particular, our techniques will use cytoarchitectural information in the form of high-resolution images of rat brain coronal sections stained for Nissl substance.

Automating brain region segmentation presents some challenges, especially at a level of granularity found in standardized atlases:

- Some regions in brain atlases are delineated based on functionality, something that a model trained on histology image data cannot exploit.
- The amount of labeled data can be a limiting factor. Training a learning model to segment all brain regions found in an atlas requires multiple, manually annotated, experimental sections for each level of the atlas.

Our work does not aim to segment brain regions based on functionality, since we only use image data, but we hope to exploit cytoarchitecture for segmentation. Our work also

does not aim to segment all brain structures present in an atlas. The scope of our work only includes a few levels and seven structures.

1.3 Thesis Statement & Research Questions

Cytoarchitectural features are present in high resolution images of Nissl-stained coronal sections of the rat brain and can be detected via computational methods. These features can be used to accurately parcellate brain structures via a computer at a granularity level present in standardized atlases of the rat brain.

Our work aims to answer the following questions:

1. Is it possible to segment rat brain regions with only information found in images of coronal sections stained for Nissl substance?
2. How does image resolution affect a computer program's ability to segment brain regions?
3. How can we utilize information at different scales to segment rat brain regions?
4. Does additional information about anatomy or physiology enable or improve segmentation of rat brain regions?
5. Is it possible to segment rat brain regions when there is no parcellation available?

1.4 Outline

The work is presented in the following order. **Chapter 2** introduces procedures for neuroscientific tract tracing studies including image data acquisition, parcellation, and registration to a standardized atlas, as well as previous work presented in the literature and introduces several segmentation methods and deep learning models. **Chapter 3** presents

the objectives, and the data and methods we will use to achieve said objectives. **Chapter 4** presents our preliminary results and a discussion about our current work. Finally, **Chapter 5** details the proposed work for my dissertation defense and publications with a timeline to complete it.

Chapter 2

Background

This chapter presents background information necessary to understand the scope of our research.

2.1 A Pipeline for Tract Tracing Studies

As previously mentioned, our work intersects with the preliminary step of delineating rat brain regions. In order to provide context, we can consider one example of a downstream task: tract tracing studies. The following sections will focus on the parts of the pipeline pertaining to image data acquisition, parcellation, and mapping to standardized atlases.

2.1.1 Obtaining Image Data for Parcellation

Brain parcellations are rendered to locate regions of the brain. In particular, researchers investigating an object in the rat brain, such as a molecule, need to identify the location where the object is found. Usually, the object can be visualized by injecting a reagent or marker into the brain which binds to the object in question. However, visualizing the reagent on its own makes it difficult to know where the object is located in the brain. Ergo, an additional non-specific stain is used to visualize landmarks in the tissue. Often, cresyl violet acetate or thionin is used as a non-specific stain since it stains for Nissl bodies, a substance found anywhere in the tissue where protein synthesis takes place. In fact, the atlas used in our work was developed by considering cytoarchitecture or cellular composition revealed by thionin staining [42].

In brief, after the rat has been injected with the marker, the rat is transcardially perfused