Coordinate System for the Cortical Laminae

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1 Introduction

The human cortex consists of 6 layers, the distribution of various neuronal cells and their properties along the layers varies between different cortical areas. Irregularities in the microstructure in the different layers or laminar of the cortex is seen in a number of neurological diseases, such as epilepsy and schizophrenia [1, 2].

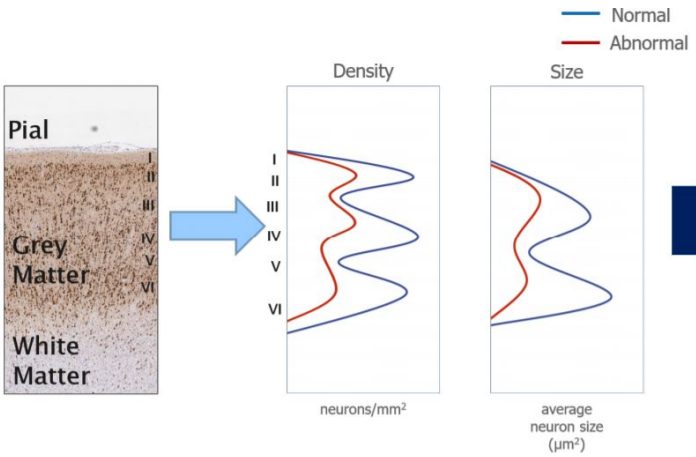

Figure 1: Histological illustration of the 6 layers of the cortex and the intricate distribution of neuronal between layers. Images on the right show the normal and abnormal profiles for neuron density and average neuron size. In general, the cortex is divided into six layers: **1)** Molecular layer, **2)** External granular layer, **3)** external pyramidal layer, **4)** Inner granular layer, **5)** Inner pyramidal layer, and **6)** Multiform layer

Currently, there is a lack of research that investigates the relationship between neuroimaging and histologically characterized laminar abnormalities. Our overall objective is to observe the correlations between different neuronal laminar features obtained in histology and scans acquired using magnetic resonance imaging (MRI). As a first step, we developed a tool or coordinate system for sampling intensities across laminae of the cortex of the brain. The tool is useful for obtaining microstructural information in histological slices, and quantitative MRI measures along the laminae of the cortex. These laminae are represented by profiles of intensities in MRI or histological measures along streamlines extending from the cortical surface to the white matter boundary. These streamlines are defined along a Laplace field, which has shown to be effective in related work [3]. Laplace's solution is defined as the solution to $\nabla\psi = 0$. The boundary conditions are defined as the grey-CSF junction and grey-white junction, with the domain of the solution defined as the individual histological slice, as described in [3]. Once, streamlines are created in histological slices, it is possible to register them into the MRI space where a direct comparison can be made between intensities of features or quantitative metrics between these two modalities.

2 Methods

Creating a coordinate system to sample intensities across the laminae of the cortex can be accomplished through the following steps:

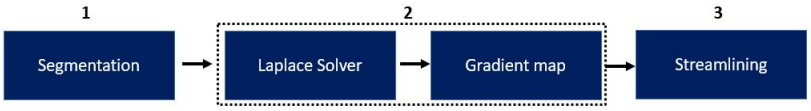

Figure 2: Work flow for creating coordinate system to sample intensities across the laminae using Laplace solution from mathematical physics.

The following sections outline in detail the methodology for each component.

2.1 Materials

The dataset consists of 20 temporal lobe epilepsy patients with temporal lobectomy. Subjects have been imaged preoperatively with structural, diffusion and functional MRI and co-registered with temporal lobe histology slices following surgical resection. Since the resected tissues is colourless and transparent, structural and cellular details are not visible unless the tissue is first stained to obtain contrast. The brain tissue in our sample were sliced and stained with NeuN, along with a number of other stains. The NeuN stain highlights the neuronal nuclei in the brain sample. Following staining, the resulting histology slides were digitized. The imaging data used to perform the analysis was a low resolution (100 micron) grayscale NeuN stained slide.

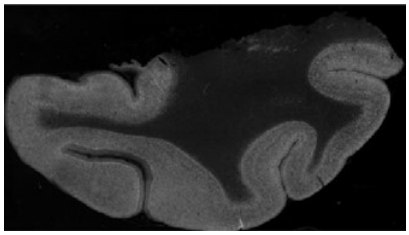

Figure 3: Grayscale image of a NeuN stained histological slice.

2.2 Segmentation

The NeuN stained histological slices were used for segmentation due to its desirable characteristics. The objective of the segmentation is to divide the grey matter, white matter, and background regions. Due to the very high abundance of neuronal nuclei in the grey matter region and the absence of neronal nuclei in the white matter regions, the NeuN stain images produces as ideal contrast for segmentation. These differences were also clear from the histogram of the NeuN stained images which showed 3 distinct peaks; ultimately these characteristics are desirable for easier segmentation.

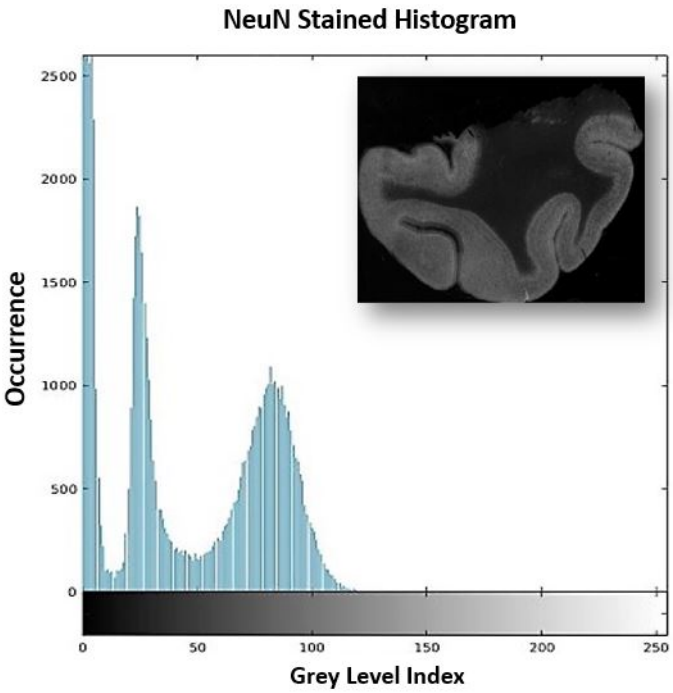

Figure 4: NeuN stained histogram, the trimodal distribution emphasize the 3 different classes or labels. in the image

The segmentation was attempted using an automated k-means clustering approach, due to its histogram characteristics. Because of a number of trade offs the segmentation was performed manually. The limitations using the the automated approach are listed as follows:

1. Segmentation from k-means cluster produced holes within different labeled regions; certain areas of the cortex are less concentrated with neuronal nuclei and may easily classified as white matter
2. K-means cluster was unable to delineate regions in between very tightly spaced folds in the cortex, also wrongly defined the first layer of the cortex as white matter due to it being lightly concentrated with neuronal nuclei.
3. K-means algorithm produced variable results with different runs for the same image.

When attempting to fill in holes using morphlogical operations, the regions between the tightly space folds would less likely be defined as the correct label. This trade off prevented us from correction of the misclassified areas. As a result of the limitations and trade offs, manual segmentation was deployed.

ITK-SNAP was used to manually segment the NeuN stained image. The following steps were taken to obtain segmentation results:

1. A outline of grey matter region was drawn, which labeled the closed region within the outline as the initial label
2. Once GM region was labeled a paint brush was used to label the WM region
3. Similarly we obtain the background segmentation through paint brush tool in ITK SNAP.

2.3 Laplace method in the cortex

Segmentation of the NeuN stained image allows us to define the boundary conditions for the Laplacian equation. The boundary conditions are defined as the GM/background junction and GM/WM junction, with the domain of the solution as the NeuN stained slice. The boundaries were determined using morphological operations from the segmented image. Explicitly, we define the following structural element:

$$\begin{matrix} 0 & 1 & 0 \\ 1 & 1 & 1 \\ 0 & 1 & 0 \end{matrix}$$

Figure 5: 3x3 structural element used to detect GM/Background and GM/WM junction.

The structural element was than used to dilate the binary image in which the white matter was considered the foreground object. Subsequently, a logical 'and' operation was performed in junction with the binary image which the GM region is considered the foreground object. The overlapping region between the dilated WM and GM region defines the GM/WM junction. It should also be noted that the Background/GM boundary was obtained in a similar fashion.

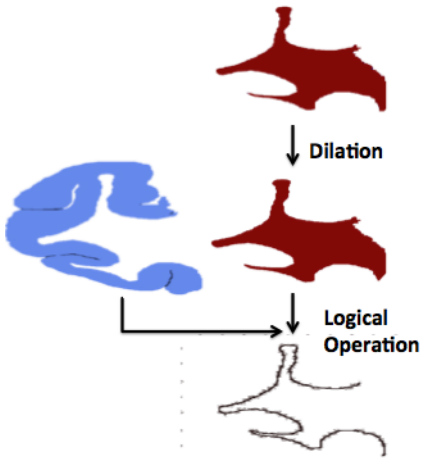

Figure 6: White matter boundary detection work flow. Red label repersent the WM segmentation and light blue label is the GM segmentation.

To solve the Laplacian equation we used open source software from the following git repository: https://github.com/jordandekraker/HippUnfolding/blob/master/matlab_src/dependencies/laplace_solver.m. The Laplace solver works only with 3D volumes, therefore we added an empty slice to the image to simulate 3D volume. Once solved Laplace's solution will produce regularly spaced surfaces or potential lines between the two boundaries. Normalized gradient of ψ form a vector field representing tangent vectors along field lines connecting both boundaries. The advantages from forming streamlines from these vector field is:

1. Mapping is 1:1, every point on GM/WM boundary is mapped to background/GM boundary and no point has more than one point mapped to it.
2. Reciprocity such that pair of points are same whether you start from either boundary.
3. Provide streamlines that are smooth and which have minimum distance between boundaries
4. Streamlines never cross in this particular application.
5. Any and every point in the interior of boundaries have a unique streamline
6. Every streamline is associated with a length which defines the thickness of the cortex.

These desirable characteristics provide motivation for using such a method to produce a coordinate system for sampling cortical laminar.

2.4 Profile Delineation

The gradient of ψ will produce a vector field that smoothly connects points from both boundaries, with the desirable characteristics described above. Streamlining will produce curves that are tangent to the vector fields by interpolating connected vectors. In order to create streamlines, it is necessary to provide the set of points the streamlines start at. The start point's were defined as the background/GM and GM/WM boundary respectively. It was required to create streamlines for both boundaries. This was because in regions of high curvature for one side of the boundary there is many endpoints and very little start points, producing highly spaced streamlines. To overcome this problem we Streamlined from starting from both boundaries.

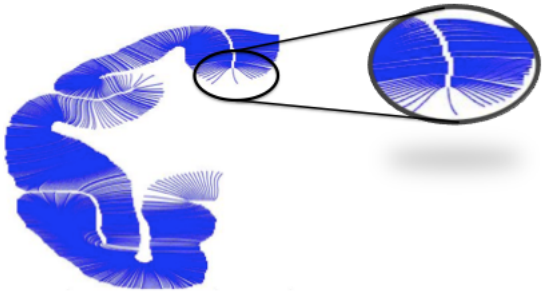

Figure 7: Streamlines starting from the GM boundary. In regions of high curvature, where there is not enough start points the streamlines are highly spaced.

3 Results

3.1 Segmentation

Initially we aimed to have an automated segmentation algorithm, however there were few limitations that provided nonnegotiable results. These limitations were discussed in section 2.2. In general white matter region is over segmented using the automated approach. Another limitation with the k-mean cluster segmentation approach was that it provided variable results across iterations.

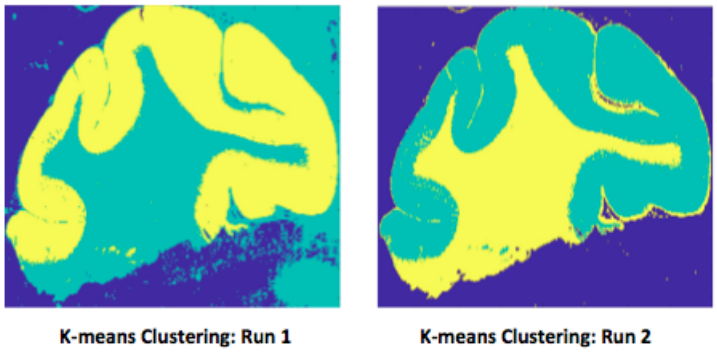

Figure 8: K-means cluster segmentation results across 2 iterations.

Due to the limitations in the automated segmentation approach, a manual segmentation was obtained for the purposes of this project.

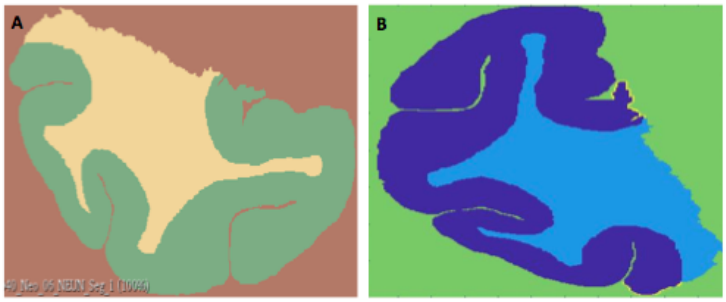

Figure 9: $\boldsymbol{A}$) Maneul segmentation viewed on 3D Slicer $\boldsymbol{B}$) Segmentation viewed on MATLAB, note when viewing in matlab there is a rotation. In image $\boldsymbol{B}$ we have a yellow label which represent regions to ignore, in total there are 4 labels in the Manuel segmentation.

3.2 Laplace

The solution to Laplace will produce finely spaced equipotential lines. The following image illustrates the solution to laplace's equation on the NeuN stained image. The changes in the color shows the gradient changes in the equipotential lines.

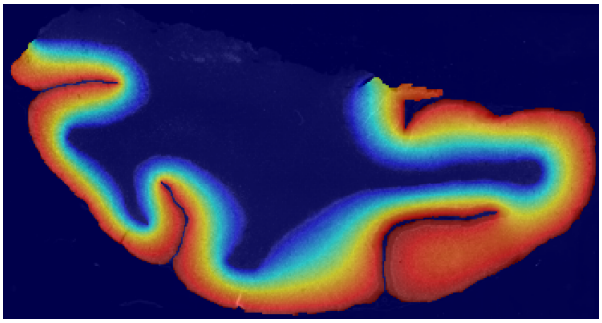

Figure 10: Laplace's solution on the NeuN stained image displayed with a heat map.

Once the solution of ψ is determined, we can determine the gradient of ψ which will produce a vector field that smoothly connects points from both boundaries.

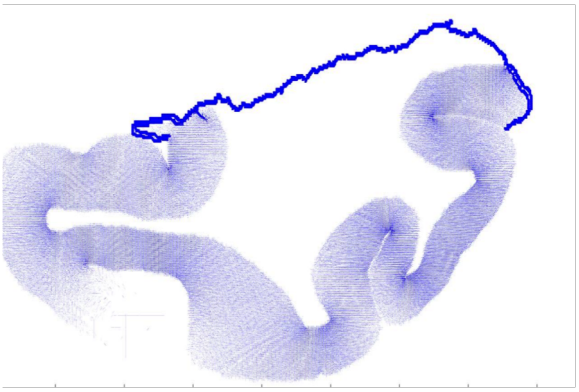

Figure 11: Vector Field from the gradient of ψ

3.3 Streamlines

Once the vector field is obtained, we can create curves that are tangent to the vector field extending from the boundary specified to the end boundary. We refer to the streamlines or curves as profiles. Profiles provide us with a tool to sample the intensities along the depth of the cortex. Complete set of streamlines or profiles are shown in Figure 12.

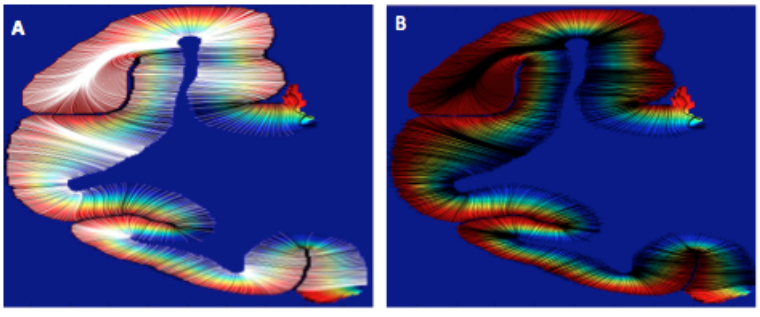

Figure 12: Streamlines overlayed onto the solution of Laplace's equation. $\boldsymbol{A}$) illustrates the streamlines starting from the pial or grey matter surface in white and starting from the white matter boundary in black. Defining streamlines from both side of the boundary provides densely spaced streamlines in all regions. $\boldsymbol{B}$) illustrates the densely spaced streamlines all in the color black.

To sample intensities along the depth of the cortex, we can sample the points along an individual streamline or profile.

4 Conclusion

In conclusion, we have provided a tool for sampling cortical laminae, this tool can be used to sample both histological and MRI samples. Currently, there is a lack of research that investigates the relationship between neuroimaging and histologically characterized laminar characteristics. Directly comparing two modalities will allow us to better understand the information presented to us in in-vivo imaging; in particular MRI. This tool provides a way of directly comparing intensities of feature or quantitative metrics between these two modalities.

Once these streamlines or profiles are defined sampling intensities along the depth of the cortex can be accomplished through sampling the points along an individual streamline or profile. Currently, the work flow to create a coordinate system for sampling cortical laminae is broken down into 3 components: segmentation, solving Laplacian equation, and streamlining.

The segmentation was performed manually due to limitations in the automated segmentation approach proposed. Manual segmentation is a very time consuming process. In order to save time, future segmentation's will become semi-automated. We will run k-means cluster segmentation as an initial segmentation, and refine the segmentation manually using ITK-SNAP. A future project can be to fully automate the segmentation process; one approach can be to use machine learning or deep learning to segment the different labels in the histological slice using a supervised approach from the manually segmented labels.

Solving laplace's equation provides us with a mathematical tool for creating streamlines or profiles for sampling the cortical laminae. In other word, it allows us to create a coordinate system for sampling the cortical laminae. Creating streamlines from laplace's equation is a common approach in literature [4]; however there have been other techniques that have been suggested to provide a more anatomically correct model [5]. In this project we are not overly concerned with the anatomical correctness for our coordinate system; rather a tool to sample and compare the two modalities.

References

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