Techniques for Extracting and Analyzing Axon Paths in 3D Images of Mouse Optic Nerves

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THE MIAMI PROJECT TO CURE PARALYSIS

Motivation

Nerve regeneration is an active area of research in the medical community. In order to test the effects of various treatments on regeneration, researchers at the Miami Project to Cure Paralysis use mice optic nerves as a model for neuron growth in general. After crushing mice optic nerves, subjecting them to various treatments, and letting them grow, they obtain 3D images of the optic nerves, specifically of the axons within the nerves. They then seek to analyze the axons, as axons in normal nerves grow in straight paths, but injured nerves feature, wavy, deformed axons.

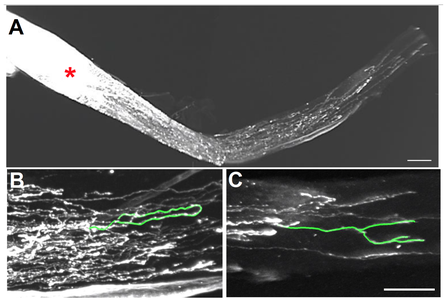

Figure 1: Regenerating retinal ganglion cells take complex courses. **(a)** A mouse optic nerve was crushed at the red asterisk. Regenerating axons are labeled with a fluorescent tracer. **(b)** A manually traced regenerating axon takes a wavy course and makes a hairpin turn back towards the retina. **(c)** A manually traced regenerating axon branches and rebranches as it grows through the optic nerve. Modified from Fischer et al, Exp. Neurol. 2017 296:83-88.

To analyze the axons, researchers must manually trace the axons, a highly tedious and time-consuming task. Factors such as noise in the images and intersecting axons make this task exceptionally difficult for both humans and computers. The purpose of this project is to develop software that automates or substantially reduces the amount of human involvement in the process of tracing the paths of the axons. Subsequently, we would use those tracings to analyze the paths the axons take, identifying features such as branches and quantifying the deformity of the axons. To do this, we would develop a pipeline that starts with a 3D image and ends with analysis of paths of axons in the iamge, with several intermediate steps.

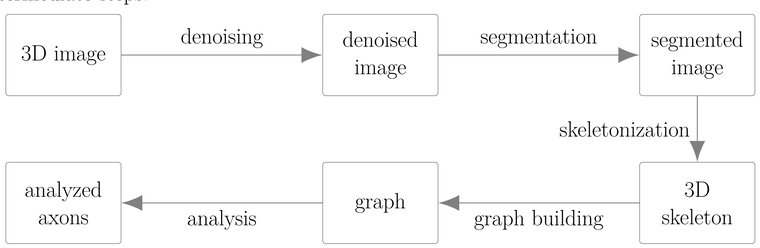

Figure 2: The proposed pipeline from a 3D iamge to analyzed axon paths.

Denoising

- Images are noisy due to imperfect optics and uneven diffusion of marker in axons.
- Simple denoising algorithms blur image, removing small, thin features such as axons.
- Nonlocal means denoising [2] is a more sophisticated denoising algorithm that preserves small features by doing global, not local averaging of voxel values.

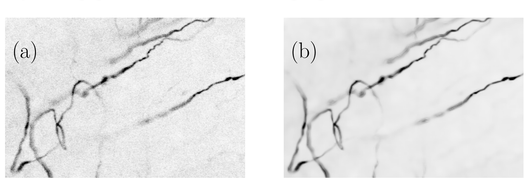

Figure 3: **(a)** A subset of one slice of an image. **(b)** The nonlocal means denoised version of that subset.

Segmentation

- We tried several existing segmentation algorithms.
  - Thresholding based on intensity value picks up too much background noise while not having axons be sufficiently connected.
  - Graphcut [1] improved upon thresholding by picking up less noise and having axons be more connected, but was not satisfactory and was much slower than thresholding.
- Standard segmentation algorithms fail because they fail to take into account information about the structures they segment. Axons are thin and elongated, and humans use this information when manually segmenting.
- We devised our own segmentation algorithm that takes advantage of this information.

Enhancing Thin Structures

- Examine the volume in a series of small windows: large enough to capture axons and background, but small enough so that axons are locally fairly linear.
- Fit a multivariate normal distribution to the window and compute its mean and variances.
- Compute the ratio of the variances along the first and second principal axes of the distribution. A high ratio means the window contains a thin, elongated structure in the direction of the first principal axis.
- Increase the intensity of voxels near the mean of the distribution based on the ratio of variances. If there is a higher ratio, the window is more likely to contain an axon and thus should be enhanced more.

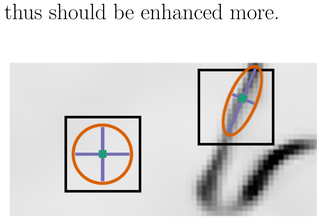

Figure 4: In a window with no axons, the distribution has roughly equal variances in all directions, so there is little enhancement. In a window with an axon, the distribution has more variance in one direction, so points around the window's mean are enhanced.

- Thresholding the enhanced image resulted in less background noise being detected along with more plentiful axons that were better connected.

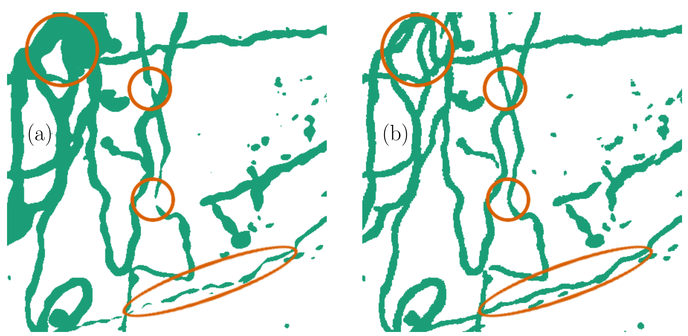

Figure 5: **(a)** A denoised image segmented via thresholding alone. **(b)** An enhanced version of that same image thresholded at a level that produces a similar amount of background noise, but with fewer lost features and more highly connected axons.

Skeletonization

- A skeleton of a 3D object is a topological representation of its features consisting of one voxel wide segments.
- Skeletons contain all information about axon paths while being easier to work with than a segmented volume.
- We used an existing skeletonization algorithm [4] that builds a skeleton by progressively thinning a shape until all features are one voxel thick.

Graph Building

- We built a graph to represent the paths and intersections between paths of the skeleton.
- We removed imperfections of the skeletonization algorithm by filtering out endpoint paths with less than a certain length and by removing vertices with only two edges.

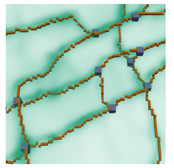

Figure 6: A skeleton and its corresponding graph structure. The original volume (green) can be represented by a skeleton, which can be represented as a graph with edges representing paths in the skeleton (orange) and vertices representing intersections of paths (blue).

Future Work: Path Detection and Analysis

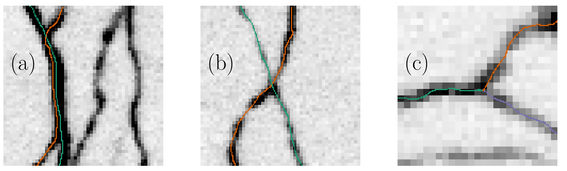

Figure 7: **(a)** Axons grow alongside eachother for a short segment due to cell adhesion. **(b)** Axons grow so close to each other so as to appear to intersect, forming an apparent four-way junction. **(c)** An axon that branches into two axons.

- Axons often grow alongside each other due to cell adhesion, and frequently intersect.
- We need to distinguish those intersections from true branches: when one axon splits into two axons that grow in different directions. To do this, we will examine the directions that segments go in at vertices.
- We also need to quantify the tortuosity of the axon paths. There are several measures that could perform this.
  - Ratio of path length to distance between endpoints.
  - Integrate some approximation of local curvature along the path.
  - Compute fractal measure of path by measuring length using segments of various length.

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