Retinal Axon Tracing Research in BIOF 350: Image Analysis

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Introduction to Retinal Image Analysis

The retina in mammals consists of two principle layers of neurons, separated by plexiform layers composed of connections between neurons. The outer plexiform layer contains connections between photoreceptors (rods and cones) and bipolar cells in the inner nuclear layer. In collaboration with Dr. Peter Fuerst at the University of Idaho, image analysis tools from LCSC are being used in analysis of these connections. One missing piece of information is the connections from photoreceptors to bipolar cells. Tracing an individual axon from a photoreceptor to a bipolar cell takes a day or so by hand, which makes it impractical to form a complete graph of connections in images such as the overview on this poster.

BIOF 350: Image Analysis

In BIOF 350, students will learn image processing techniques, with a focus on biological applications. This covers common types of images acquired, using MRI (Magnetic Resonance Imaging), SBEM (Scanning Block-face Electron Microscopy), light microscope, and possibly LCSM (Laser Scanning Confocal Microscopy) as representative examples. About half the class will be focused on the specific challenge of tracing axons. This includes both testing potential methods and writing precise descriptions of results. The initial offering will be in Fall 2019. The class is 4 credits, two 75-minute classes plus 2-hour lab.

The Test Framework

Image processing can be done using nearly any programming language. This Summer, a C++ framework is under development at LCSC which will support using graphics processors with GLSL (GL Shader Language). Support for the new Nvidia RTX GPU is planned, and the class will include a brief primer to using a GPU (Graphics Processing Unit). In addition, a test framework has been developed by Jesse McDonald at LCSC which supports image processing operations in the Java language.

Acknowledgments

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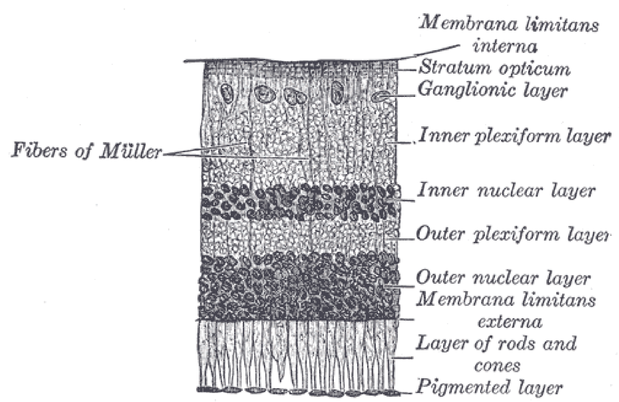
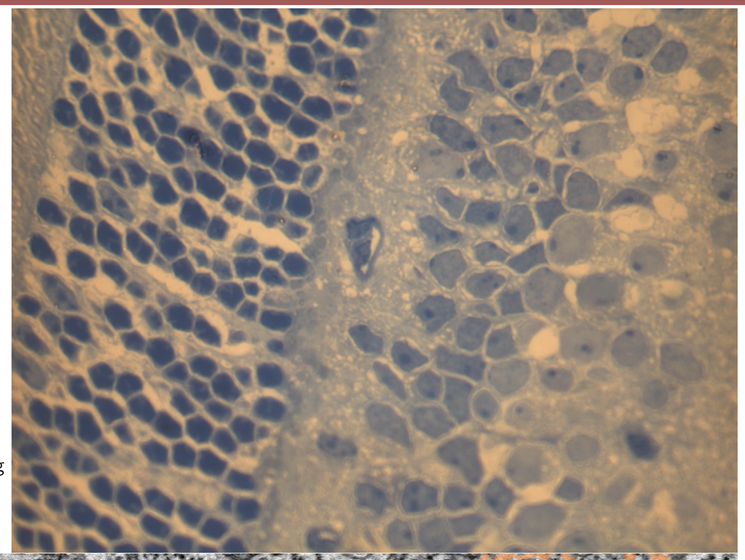
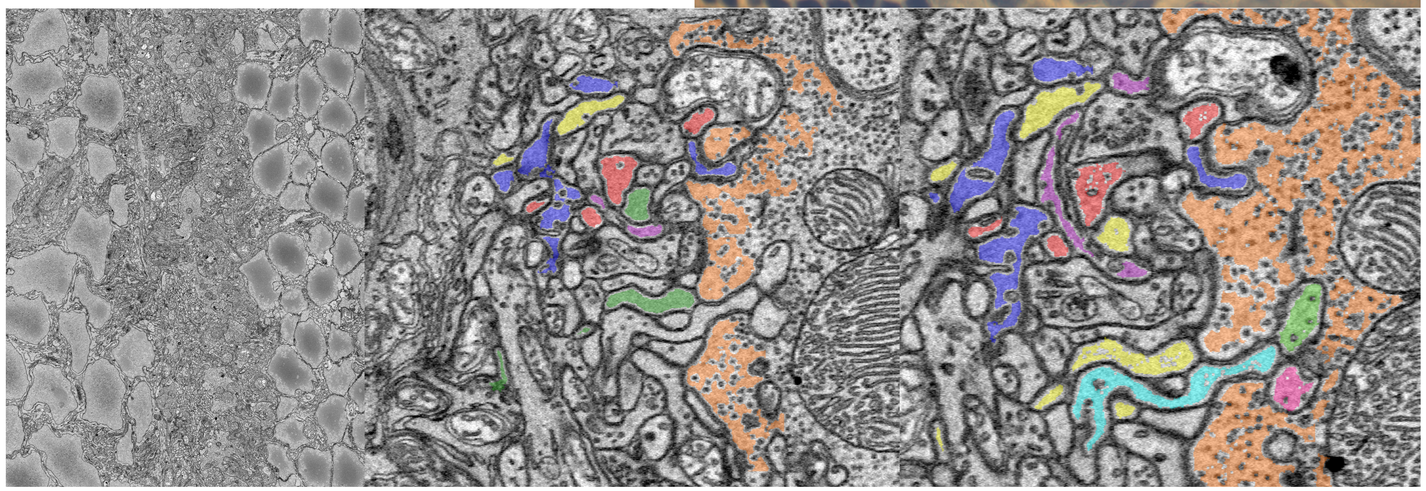
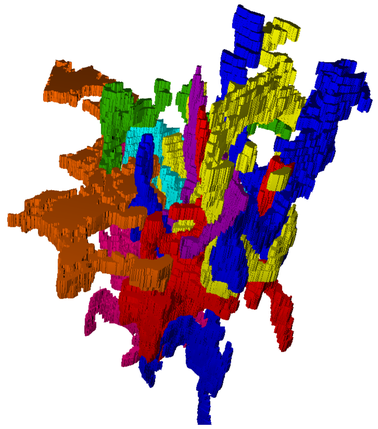
Example Images





Above: Textbook picture from Gray's Anatomy of the Human Body (1918) showing labeled layers in the retina.
Right: Light microscopy picture of the mouse retina, collected at the WSU FMIC





- Middle Row, Left: SBEM overview of the retina. This is one image out of a stack of 600. SBEM (Scanning Block face Electron Microscopy) uses a scanning electron microscope combined with a microtome to produce a stack of aligned images. Offset from one image to the next is small, but in some cases significant.
- Middle Row, Middle and Right: SBEM images with axons highlighted. Each color is only applied to a single axon. These are from the same image stack, at different levels. These were outlined with an early version of our tool, which struggled with areas containing many synaptic vesicles. This caused troubles when outlining the cone pedicle in orange.
- Left: 3D reconstruction of the structures outlined above. As above, not all structures in the image are outlined. This is a frame from a movie showing the complete rotation. Note that the thickness of each layer is much greater than the size of a pixel in a layer. As such, when treated as a 3D image, voxels are not cubes, but rather rectangular prisms.

Method 1: Area Expansion

Area expansion starts with a single voxel (similar to a pixel, but in 3D) designated inside an axon. Each of the voxel's 6 neighbors are potentially inside the axon as well. If they are part of the inside of an axon and not a membrane, than each of them are added, and each neighbor of the original neighbors is considered in the same manner, continuing until all neighbors are either part of the axon or represent an area of membrane. This method was used in 2D for the outlined images in this poster, and generally performs well. However, if the membrane is incorrectly identified at any point, it may include areas not part of the axon. Also, if the axon contains a large number of synaptic vesicles, it tends to halt axon discovery early. These disadvantages may apply less strongly in 3D.

Method 2: 3D Trajectory

As axons do not seem to generally make very sharp turns in the retina. As such, the task is to follow a series of gradual curves, with a minimum radius perhaps 5 times that of the axon itself. So maintaining a direction in 3D (heading and elevation angles) could allow an axon to be followed, with a maximum sharpness of turn defined to minimize the effect of a membrane classification error. This has the advantage of providing a straightforward means of overcoming large numbers of synaptic vesicles. However, it may struggle with sharp turns, and remains untested.

Method 3: 3D Linear Regression

At LCSC, DeLaney Jones has developed a 2D linear regression algorithm to follow membranes, which is ready for beta testing currently. Testing is planned for Summer 2019. Applying this idea to 3D imagery might be an effective way to follow an axon. Her model calculates a line based on a short section of membrane, and then attempts to find more membrane close to the line. This would be very effective on a near-straight axon. The challenge will be finding an appropriate length of line to be effective in an axon. This method may be more effective on mostly-straight axons than Method 2, but may be less effective the more sharply the axon turns. Methods 2 and 3 could potentially be used in tandem, with the results aggregated in some way to provide superior accuracy.

Method 4: RTX Light Sources

The new RTX GPU offers the chance to place a simulated light source in a 3D world. Defining the axon walls as solid, it would be possible to place a series of lights down the middle of the axons. Lights would be placed in any lit region a fixed distance from the previous light. This would require a few RTX GPUs available. We currently own 5 at LCSC, courtesy of INBRE. More will be added at some point as part of an INBRE Pilot Project which will start this Summer. Method 4 will require students who are either experienced graphics programmers, or are willing to spend extra time to learn.

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