Greater San Diego Science and Engineering Fair 2018 PROJECT SUMMARY

Name: Saeyeon Ju
Grade: 11 School: Scripps Ranch High School **Advisor:** Mrs. Gillum

Project Title: New visualization and analysis approaches using 3D electron microscopy and 3D printing technologies: Studies of optic glioma mouse model and retina microcircuit

Abstract

Objectives/Goals

Serial block face scanning electron microscopy (SBEM) is a highly advanced technology to create three-dimensional(3D) EM image stacks from 2D electron micrographs. Challenges of 3D EM have now shifted from how to capture the difficult-to-measure to what to do with all this big data. While our ability to acquire large volumes of 3D EM data is progressing rapidly, more advanced analysis tools and visualization methods are needed to assist in measuring precise 3D morphologies of organelles within data sets.

Hypothesis

1. An automatic segmentation can reduce the time spent on annotation and quantification of the high contrast mouse optic glioma SBEM dataset and allow reliable 3D visualization and quantification of axon damage and alterations. 2. A semi-automatic segmentation (IMOD interpolator segmentation) can reduce the reconstruction time of low contrast mouse retina SBEM dataset and reveal the interaction between retinal ganglion cells (RGC) and bipolar cells via synapses. 3.Morphologies of synaptic interaction between bipolar cells and RGCs can be visualized using new trial of visualization, 3D printing technology.

Methods/Materials

In this project, the manual, semi-automated, and auto-segmented 3D reconstructions are tested in the analysis of variable contrast SBEM dataset. Manual segmentation has performed with closed contour and drawing tools in IMOD and semi-automatic segmentation has performed with the "Interpolator plugin". Automatic segmentation has applied combination imaging tools used by imodauto and ImageJ. Lastly, 3D printing is performed with Autodesk Meshmixer and Ultimaker Cura to create the retina microcircuit model.

Results

Automatic segmentation remarkably reduced annotation time in mouse cancer SBEM data, with the time spent for manual segmentation for about a hundred axons being about 40 hours. However, the automatic segmentation time spent was 30 minutes to an hour. Auto-segmentation analysis clearly addressed the alteration and degeneration of the axon in the optic glioma. In the glioma model, each axon diameter was smaller than a WT axon and the volume of the axon was significantly reduced. Revealing the retinal neuron microcircuit between RGC and bipolar cells is quicker due to alternative semi-automatic segmentation. The segmented RGC identified to a bistratified RGC whose dendrites stratify with both ON and OFF sublamina and the rod bipolar cell made direct synaptic connections with the RGC in the ON-sublamina which was not thought to directly synapse onto ganglion cells. Due to the limitations of printer technology, the delicate morphologies of retinal neurons pose the main technological challenge to constructing these 3D printouts; in my project, the reconstructed 3D retinal neuron model files successfully produced the retinal microcircuit 3D printing model.

Conclusion/Discussion

Automatic segmentation with 3D EM dataset is a strong tool to diagnose illnesses of the optic glioma 3D EM, a relatively high contrast dataset. This technique should be extended to segment myelinated axon boundaries in brain images, where the axons do not follow the same direction and the staining is not limited to myelin sheaths. IMOD interpolator segmentation in 3D EM data is another advanced tool to accelerate the reconstruction of low contrast datasets. Type of retinal neurons and their synaptic interaction has addressed in shorten time. Thus, these tools fill a critical need by allowing for the quantitative analysis of volumetric EM datasets at a nanoscale. The combination of the optimized annotation technique with 3D EM datasets and 3D printing can obtain high-resolution morphological data for microcircuits.

Summary Statement

The manual, semi-automated and auto-segmented methods are tested in the mouse optic glioma and retina SBEM dataset to reveal the advantages and limitations of the software programs, and 3D EM technologies and 3D printing visualization are combined to display these synapse-level models and their connectivity.

Help Received

Mrs. Gillum, Drs. Guy Perkins, Keunyoung Kim and Scott Mcavoy