Software for the Analysis of 3D Biological Data Sets

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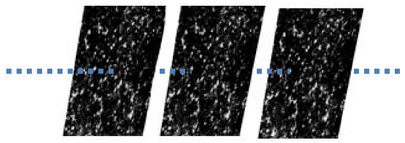
Abstract

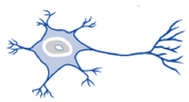
This project is the creation of a software interface and data processing toolbox tailored to the specific needs of a neurobiology research group. The project scope and requirements were set in collaboration between Cornell University's Electrical and Computer Engineering Department and Cornell's Department of Neurobiology and Behavior. The data set and characterization process are essential to the research conclusions of the Harris-Warrick group in Cornell's Neurobiology Department. The data set is a sequence of greyscale 2D images taken from a confocal microscope at different depths. The process of characterizing the data set involves segmentation according to intensity and geometric shape within a unique noise environment imposed by the confocal microscope. Prior to my project's completion, the research group was only able to perform this analysis manually in 2D. The application of image processing techniques to this biological context provides value to the researcher by improving the precision and accuracy of measurements as well reducing the time and frustration encountered through manual data characterization.



3D Data Set

Background

Professor Ron Harris-Warrick's lab is investigating the cellular consequences of spinal cord injury (SCI). As part of their research, they have begun to analyze the SCI-induced changes in sensitivity to serotonin (5-HT) by identifying the presence of serotonin receptors. The serotonin receptors can be visualized in transverse image sections of a spinal cord through the use of a confocal microscope. The images are spaced closely together to create a three dimensional representation of a short segment of the spinal cord.

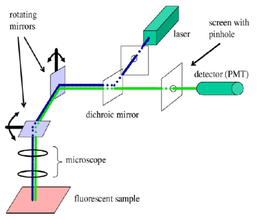


Motoneuron Model

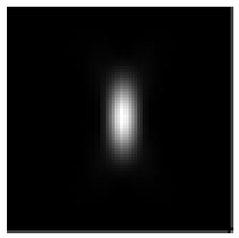
Muscle paralysis after spinal cord injury is partly caused by a loss of brainstem-derived serotonin (5-HT), which normally maintains motoneuron excitability by regulating crucial persistent calcium currents[1]. It has been observed that one of the long term effects of the lost serotonin is that the motoneurons compensate by becoming more sensitive to serotonin to regain excitability. In an effort to better characterize the increase in motoneuron sensitivity, the Harris-Warrick lab is monitoring the long term changes in overall number, size, and intensity of 5-HT_{2C} receptor sites in healthy mice and mice with a spinal transection.

The resolution of the confocal microscope is limited by the diffraction of light. When the fluoresced light is passed through the circular pinhole aperture it diffracts in a pattern that is referred to as the Point Spread Function (PSF).

Confocal Microscope Diagram



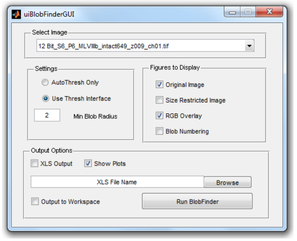
Confocal Microscope PSF



2D Serotonin Receptor Identification

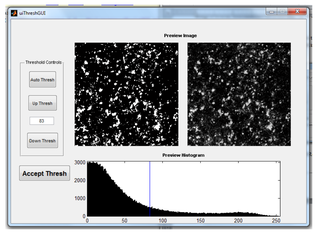
User selects:

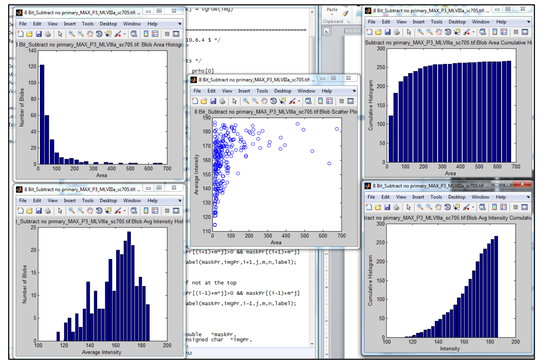
- Minimum Allowable Blob Radius
- Interactive Thresholding
- Output method – MATLAB workspace or Excel
- Output graphs and clarifying figures

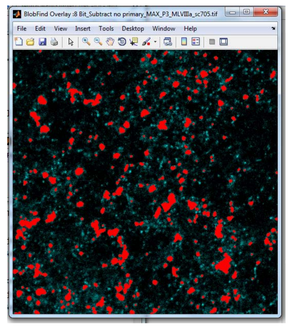


Software uses:

- Morphological open to restrict blob size
- Connected Component analysis in 2D to segment blobs







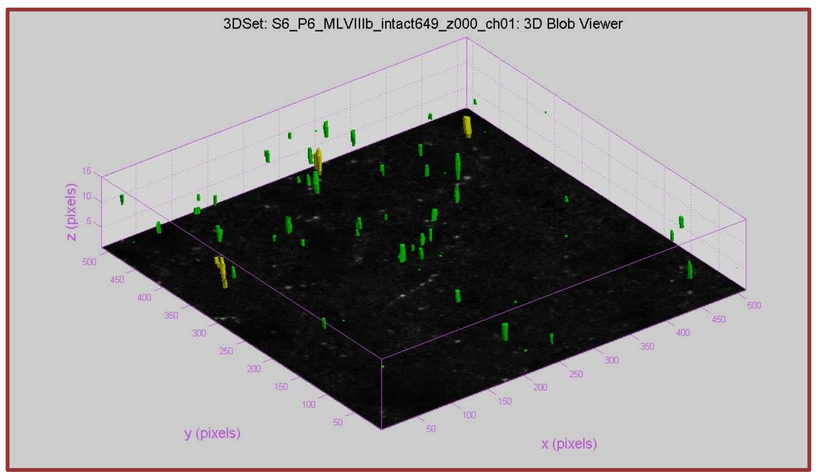
3D Serotonin Receptor Identification

User selects:

- Minimum Allowable Blob Radius
- Interactive Thresholding
- Output method – MATLAB workspace or Excel
- Output graphs and 3D rendering

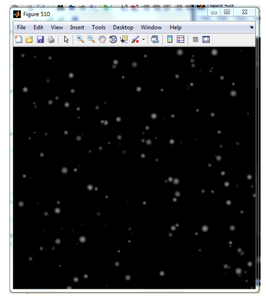
Software uses:

- Morphological open to restrict blob size
- Connected Component analysis in 3D to segment blobs
- Isosurface patch calculation



Testing and System Qualification

In order to qualify the methods used above, it was important to create a known data set for the 2D and 3D setups. I wrote a script to populate a 3D volume with a specified number of spheres of a specified radius and intensity gradient in order to qualify the techniques and 3D rendering with a known data set and dimensional aspect ratio. Slices of this populated data set were used to test the 2D tools.



Results

The end product of this project is fully functional to the requirements asked by the neurobiology lab and has surpassed their expectations. More importantly, the software has already aided in the research conclusions of an undergraduate researcher, Gabrielle Van Patten, as part of her Research Honors program thesis. I expect that the tools provided by this project will be useful in further confirming the lab's receptor sensitivity hypothesis in future publications.

Future Considerations

In an effort to cmake the software reated for this project most usable for the neurobiology in future work I made the infrastructure modular so implementing new functionality is simple and comes with instructions in the User's Manual. If, for example, they wanted to add functionality for characterizing the web-like Calcium channels they could simply write the script for it, connect it to the menu, and implement the function I/O as per my instructions in the User's Manual.

References

1. Murray, Katherine C., Aya Nakae, Marilee J. Stephens, Michelle Rank, Jessica D. Amico, Philip J. Harvey, Xiaole Li, R Luke W. Harris, Edward W. Ballou, Roberta Anelli, Charles J. Heckman, Takashi Mashimo, Romana Vavrek, Leo Sanelli, Monica A. Gorassini, David J. Bennett, and Karim Fouad. "Recovery of Motoneuron and Locomotor Function after Spinal Cord Injury Depends on Constitutive Activity in 5-HT_2C Receptors." *Nature America*16.6 (2010): 694-701. Print
2. Hutcheon, B., L. A. Brown, and M. O. Poulter. "Digital Analysis of Light Microscope Immunofluorescence: High-resolution Co-localization of Synaptic Proteins in Cultured Neurons." *Journal of Neuroscience Methods* (2000): 1-9. Print.
3. Prasad, V., D. Semwogerere, and Eric R. Weeks. "Confocal Microscopy of Colloids." *Journal of Physics: Condensed Matter* 19.11 (2007): 113102. Print.

Acknowledgements & Contact Info

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