Deep Learning-Based Neuron Detection in Brain CLARITY Imaging

Project Proposal

Students: Prerna Singh

Mentors: Dr. Jeremias Sulam

Objective: To develop a robust 3-dimensional convolutional neural network (CNN) that can predict, with increased precision and accuracy when compared to other models, how many fluorescent neurons are present within a section of a brain imaged with CLARITY.

Background:

Clear lipid-exchanged acrylamide-hybridized rigid in-situ-compatible tissue hydrogel (CLARITY) imaging enables "lossless high-resolution brain-wide imaging" through the use of tissue transparency techniques.[1] The CLARITY imaging technique is unique in that it makes brain tissue transparent, allowing for a complete 3-dimensional representation of the brain in a 2-dimensional scan. This tissue-hydrogel technique allows for imaging of the most internal parts of the brain, giving a new perspective on the biology of the brain.[2]

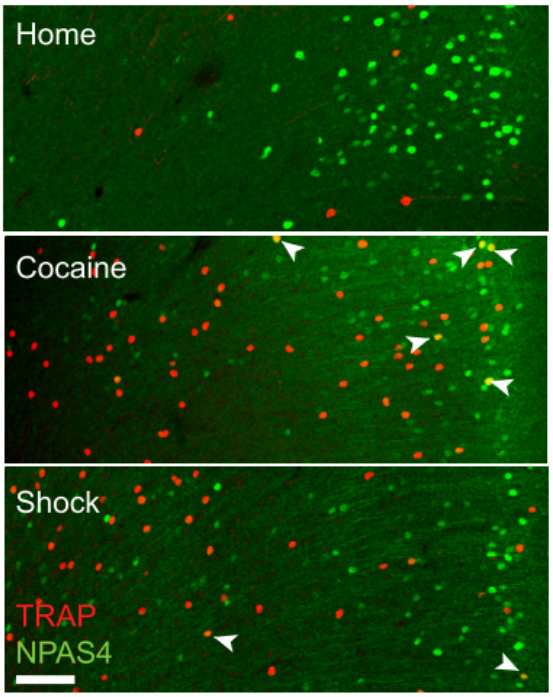


Figure 1 depicts the fluorescence where a specific antibody is binding to a cell in a mouse prefrontal cortex. The different colors represent different cell types: TRAP and NPAS4, which can all be differentiated in these scans.[1]

More generally, specific antibodies can be engineered to bind to cells and cause fluorescence, based on which cells scientists are studying within the brain. Being able to look at the brain in three dimensions allows scientists to focus on certain structures without losing focus of the whole brain in the background.

Figure 1: Image from Wiring and Molecular Features of Prefrontal Ensembles Representing Distinct Experiences, Ye et al., 2016, Cell 165[1]

Motivation:

CLARITY is a critical tool for the connectome project[5], which looks at how neural cells fire together, within a hierarchy of neural activation patterns in the brain. The overall goal of the connectome project is to develop a comprehensive map of neural connections in the brain. CLARITY can give a lot of insight into that by seeing how different parts of the brain activate and fluoresce with neurons in other locations in the brain, which has not been possible given the intrinsic limitations of previous kinds of imaging, which give only a 2-dimensional picture of cells in the brain.

CLARITY can also help in the understanding of neurotransmitter pathways by using antibodies that bind to neurotransmitters, their precursors and end products, and tracing their location in the brain over time. Lastly, CLARITY can help in studying neurological diseases, by focusing on

diseased or damaged structures in the context of the brain as a whole, where previously, only small 2-dimensional sections of a specific brain structure could be studied like this.

The CLARITY scans used in this project are focused on the connectome project. The experiments try to understand how different cell types in the brain link their activity and pathways. Through CLARITY scans, cellular logic and behavior can be understood through cell-type specific excitation throughout the brain.[1]

In any of these different applications of CLARITY, processing each scan usually entails annotating and counting cells by hand. These images can be fairly large, and for instance, in the data set used in this project, there are ~1000 slices per brain. In practice, this can be very time consuming and inefficient, given the amount of information that can be extracted by these scans. Previous methods attempted to solve this problem include: filtering, template matching, and blob detection, which had a maximal accuracy of ~59%. These are all non-learning methods, which asks the question if a deep learning approach would have better performance. Development of a 3-D CNN to count the fluorescing cells, will streamline the processing of CLARITY scans, allowing for critical information from these scans to be available almost immediately.

Technical Approach:

The project workflow is split into the following main components:

1. Design and Implementation of 3-dimensional CNN Model
2. First training and validation of 3-dimensional CNN training on reduced dataset (1 brain)
3. Large scale training on complete dataset (9 brains) as well as CNN validation

<u>Design and Implementation of 3-dimensional CNN Model</u>

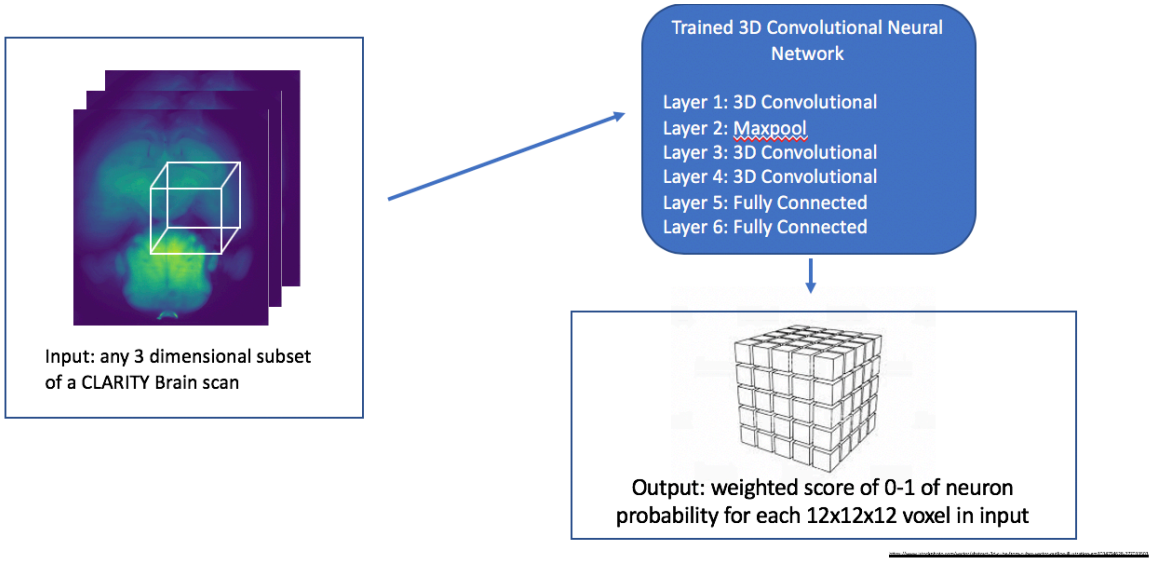


Figure 2: Shows input and output of trained 3D CNN.

The 3D CNN Layers proposed are based on the paper "Automatic Detection of Cerebral Microbleeds from MR Images via 3D Convolutional Neural Networks" that detects cerebral microbleeds in 3D brain scans and outperformed previous attempts at this problem with increased accuracy and sensitivity and fewer false positives.[3] The structure of the CNN is shown in figure 2 below. It has three convolutional layers as well as one maxpool layer, and ends with two fully connected layers. The advantage of using a 3D CNN is that it can take advantage of the spatial contextual information to extract more representative high-level features for detecting these neurons.[3]

This CNN can take an input of any size greater than 12x12x12 pixels, and reports a weighted score from 0-1 of whether it is a fluorescing neuron (1) or not (0). From here, a sensitivity threshold will be determined based on optimization of performance metrics (precision, accuracy, sensitivity, recall, etc.)

First training and validation of 3-dimensional CNN training on reduced dataset

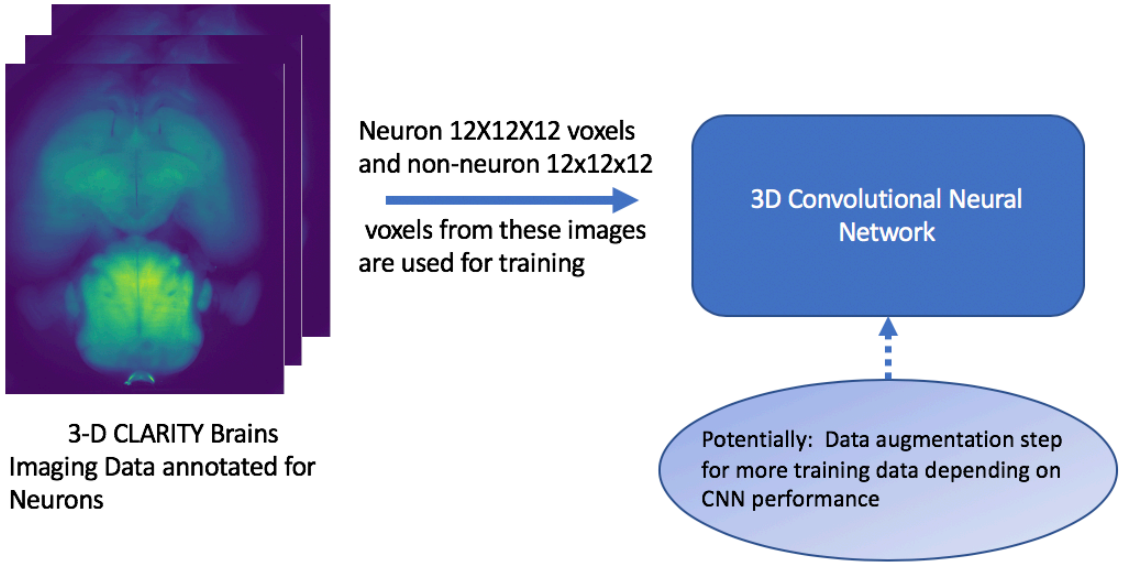


Figure 3: Workflow of training of CNN. 12x12x12 voxels are used for training, with both positive and negative controls.

The original format of the training data is CLARITY images for nine full brains. It contains ~1000 two-dimensional images, 2560x2160 pixels, taken at different depths of the brain. These nine brains contain annotations for the coordinates of some of the fluorescing cells, which include total of over 2000 annotated cells.

Based on preliminary measuring and previous work, a voxel size of 12x12x12 pixels will be used contain the entire cell, and those voxels will be the positive training data for the CNN. To generate

negative samples to train on, a similar amount of non-cell regions will be randomly generated from the other pixels not in 12x12x12 regions centered on the cell annotations.

Based on the quality of preliminary performance metrics from a reduced data set of one brain, data augmentation will be performed to attain additional training data. Examples of data augmentation are rotating the input voxel, training voxels that contain half of a cell, or off center, as well as including more voxel sizes.

Large scale training on complete dataset (9 brains) as well as CNN validation

All nine brains will be trained for completion of the final packaged model. However, in order for the validation to be unbiased, two different validations will be performed, by excluding some training data and using it for testing. The first is coarse validation, where metrics such as area under the ROC curve (precision vs. recall), precision, recall, sensitivity, and accuracy will be reported over some random splits of training vs. testing data. The second validation is full leave-one-out cross validation, where the same metrics will be reported and averaged over nine folds. In each fold, all but one brain will be used for training and the remaining one for testing.

Deliverables:

Documentation:

- Python source code
- Code documentation
- Report describing methods and achievements

Minimum: Expected 3/29

- Trained 3D CNN model (pytorch) for neuron detection
- Report in Jupiter notebook – explains training of CNN and predictions

Expected: Expected 4/20

- Robust and validated trained model (pytorch) for neuron detection
- Packaged model with documentation –made available on the internet with equal or better performance than competing simpler alternatives

Maximum: Expected 5/15

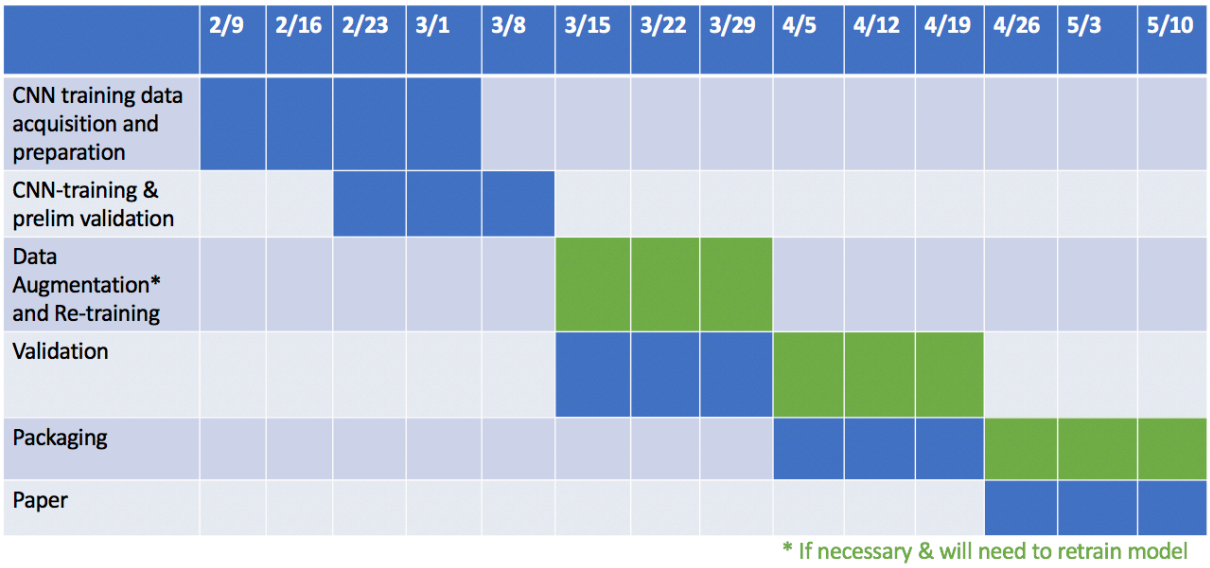
- Academic paper describing packaged model, training, validation
- Surpass baseline accuracy of previous models by a significant margin

Dependencies:

Dependency	Solution	Date Expected	Date Required	Alternative	
Computing power	MARCC Access granted through Dr. Sulam	2/10	2/25	Very slow training	✓
Computer	Personal laptop	2/10	2/10	Desktop computer provided in Sulam Lab	✓
Backups	Private github code storage,	2/10	2/25	code & data also stored on MARCC	✓
pytorch	Install pytorch for use on MARCC	2/10	2/25	Tensorflow	✓
Data- Images	Downloaded off of BOSS Neurodata	2/10	2/16	--	✓
Data-Annotations	Provided by Dr. Sulam	2/10	2/16	--	✓

ALL RESOLVED

Schedule:



In case there is a need for substantial data augmentation to attain better performance, there is potential for a small set-back shown in green.

Key dates:

3/1	CNN input script completed
3/8	Determine whether data augmentation necessary based on preliminary accuracy analysis
4/5	Model completed
4/18	Model validated

5/10	Software package completed

Management Plan:

- Meetings with Dr. Sulam every two weeks and additionally as necessary
- Code stored on github and MARCC
- Code backed up to github every week and more often with substantial improvements
- Communication through Slack/email

Bibliography and Reading List: (change to IEEE)

1. L. Ye, W. E. Allen, K. R. Thompson, Q. Tian, B. Hsueh, C. Ramakrishnan, A.-C. Wang, J. H. Jennings, A. Adhikari, C. H. Halpern, I. B. Witten, A. L. Barth, L. Luo, J. A. Mcnab, and K. Deisseroth, "Wiring and Molecular Features of Prefrontal Ensembles Representing Distinct Experiences," *Cell*, vol. 165, no. 7, pp. 1776–1788, 2016.
2. Karl Deisseroth, "Building a See-through Brain- A New Experimental Approach at the Interface of Chemistry and Biology," *Scientific American,* 2016
3. Q. Dou, H. Chen, L. Yu, L. Zhao, J. Qin, D. Wang, V. C. Mok, L. Shi, and P.-A. Heng, "Automatic Detection of Cerebral Microbleeds From MR Images via 3D Convolutional Neural Networks," *IEEE Transactions on Medical Imaging*, vol. 35, no. 5, pp. 1182–1195, 2016.
4. C. Magliaro, A. L. Callara, N. Vanello, and A. Ahluwalia, "A Manual Segmentation Tool for Three-Dimensional Neuron Datasets," *Frontiers in Neuroinformatics*, vol. 11, 2017.
5. "NIH Launches the Human Connectome Project to Unravel the Brains Connections," *PsycEXTRA Dataset*, 2009.