Segmentation of *In-Vivo* 1-Photon Neural Videos Using Neural Networks

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Abstract

Using a calcium-sensitive and fluorescent protein complex, calcium-imaging is now used *in vivo* to optically record neurons, promoting further research using this technology [1]. The two-photon modularity of the microscopy used in conjunction with calcium-imaging provides advantages such as a higher resolution, allowing for computer programs to be readily developed to identify and mask active neurons throughout a recording [2]. The lesser common one-photon modularity has a much larger field of view than two-photon, although the task of identifying and isolating neurons is inefficient; there is a clear need for an automated and accurate process finds and neurons them in one-photon microscopy. This research displays the creation of a convoluted neural network named STNeuroNet and analyzes its ability to detect active neurons in 1-photon microscopy. We find that STNeuroNet is the best computational tool to identify active neurons, although accuracy improvements may be made.

Introduction/Background Knowledge

Calcium-Imaging

Calcium ions are used for a variety of different intracellular signals across the human body, including in neurons. In addition to its abundance, calcium is also able to easily localized, making calcium a great tool for determining the location of cells [1].

To view calcium concentration optically, fluorescent proteins were combined with calcium-sensitive proteins. The protein complex used in this experiment is GCaMP, a genetically encoded calcium indicator that fluoresces when calcium is present. The combination of green fluorescent protein and calmodulin, the calcium sensitive protein, allows for researchers to view and distinguish active neurons. When calcium is present, calmodulin undergoes a conformational shift that activate the fluorescence.
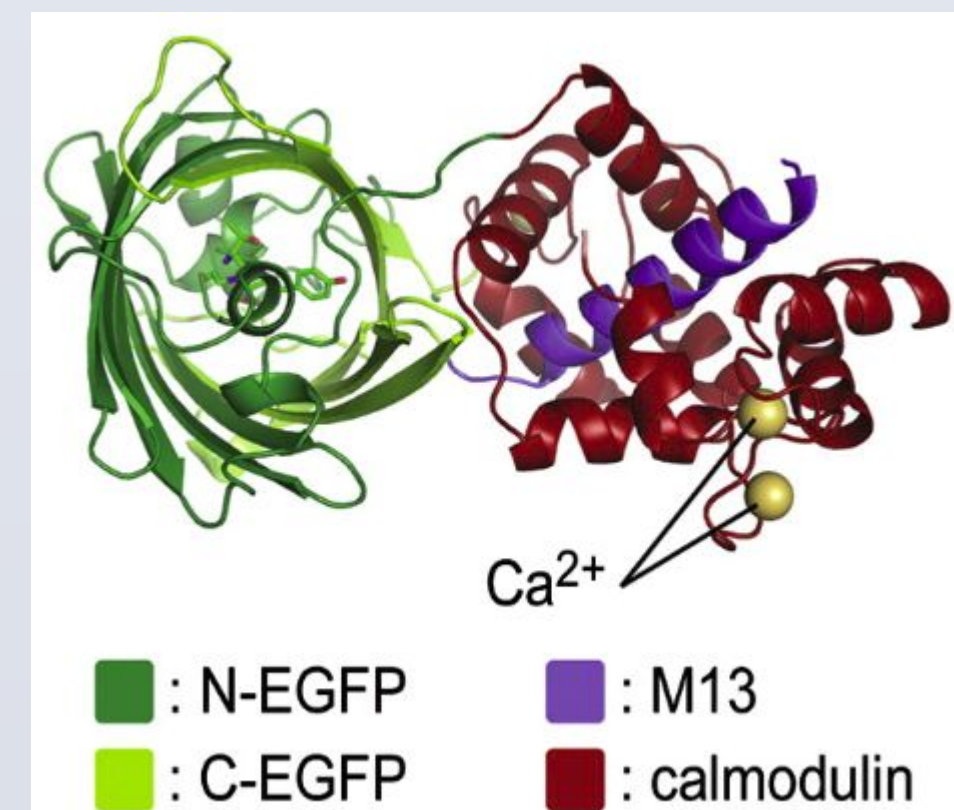

Fig. 1

3D Rendering of GCaMP protein complex. Green represents the Green Fluorescent Protein, maroon represent the calmodulin, and purple represents another connector protein, M13. [2]

Imaging Modularities

The two-photon modularity of the microscopy used in conjunction with calcium-imaging provides advantages such as a higher resolution, allowing for computer programs to be readily developed to identify and mask active neurons throughout a recording [3]. The lesser common one-photon modularity has a much larger field of view than two-photon, although the task of identifying and isolating neurons is tedious, requires the expertise of someone trained, and by extension, expensive.
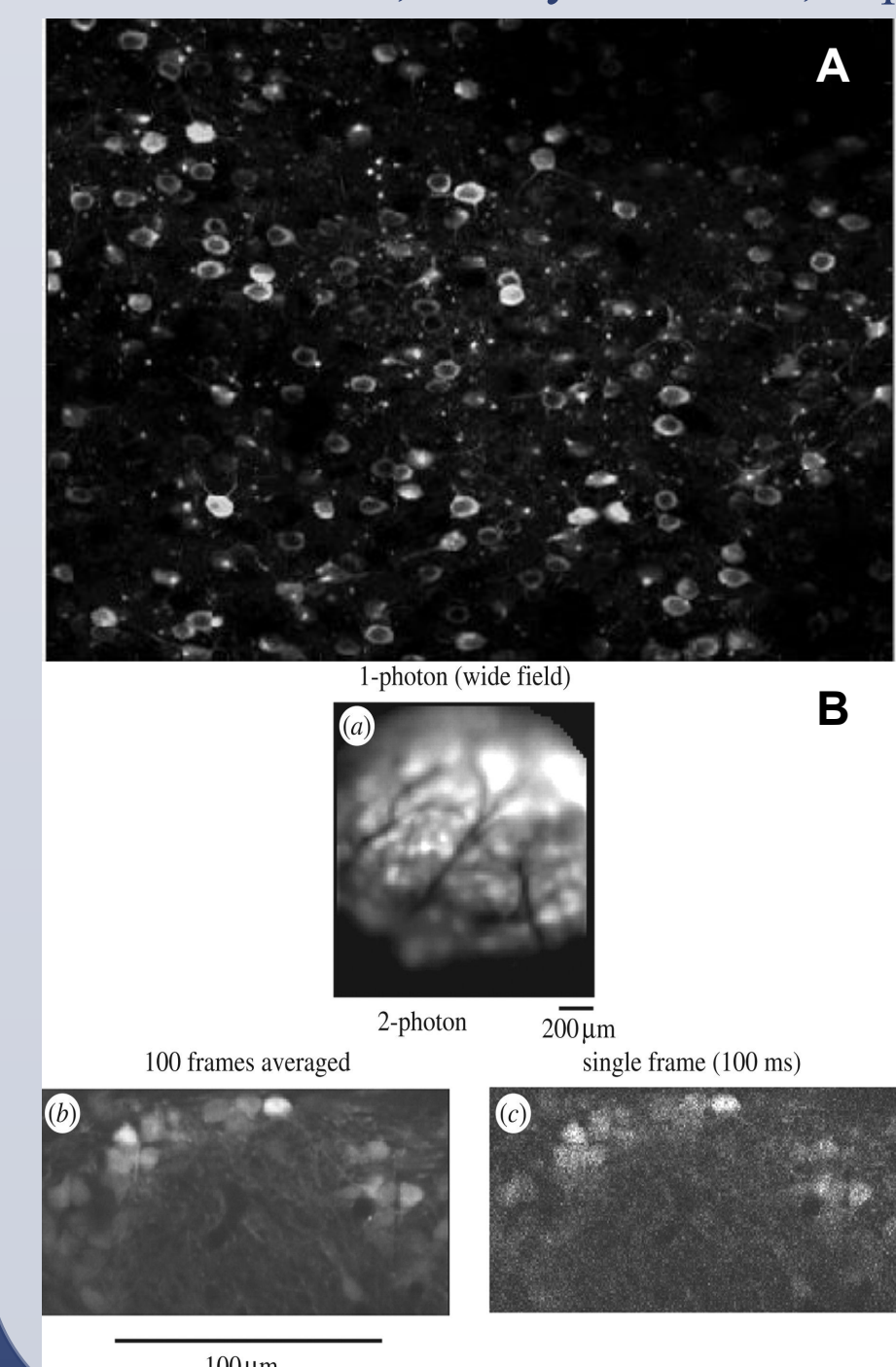

Fig. 2

A. Example of images obtained from 2-photon microscopy

B. Example of images obtained from 1-photon microscopy

Note the different in clarity between the two modularities. Images obtained from the Gong Lab.

Neural Networks

A relatively new computational method, neural networks are a type of deep machine learning that is "trained" to output specific type of output given specific input.

Consisting of individual connected nodes called "neurons", a neural network works by activating each neuron in a different way, each neuron one layer connected to the next layer of neurons in a set of numbers called "weights". Through various functions, these connections influence the activation of the following layer of neurons. This continues until the neural network approaches the output layer, where the final output is given to the user [4]
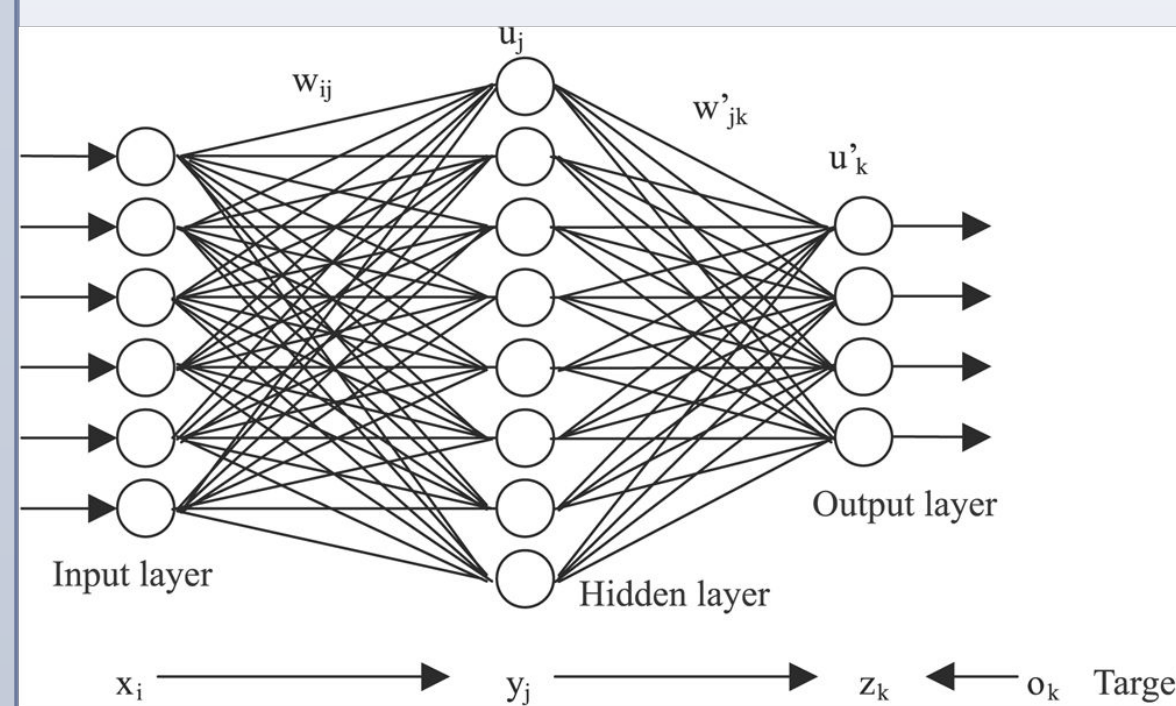

Fig. 3

Visual description of neural network with 1 input layer, 1 hidden layer, and 1 output layer [5]

During the "training phase", the neural network must be trained on how to manipulate the input data to output the correct output data. To do this, a "training set" is created for the neural network to use back-propagation techniques to "learn" to output the correct output.
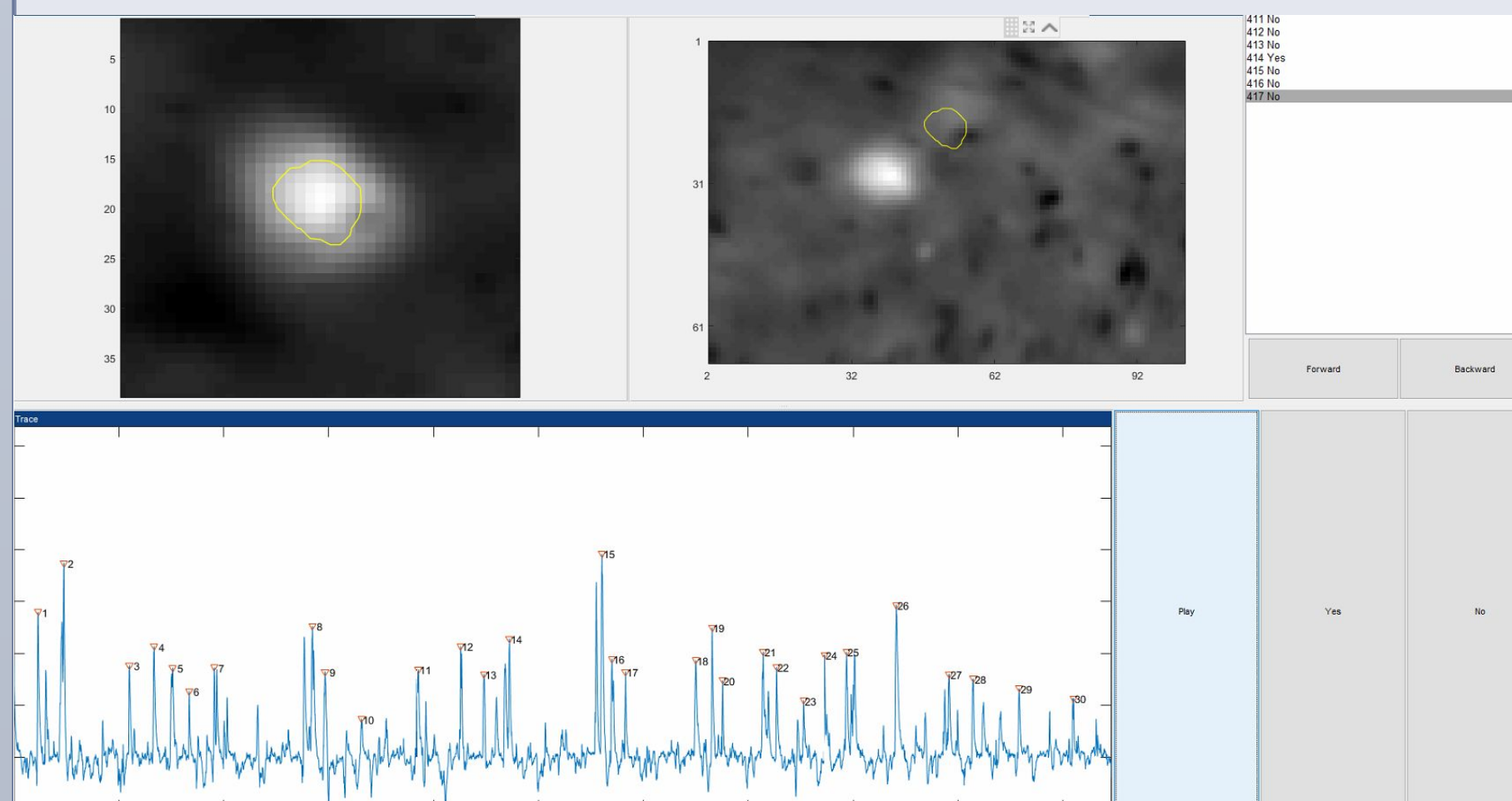

Fig. 4

Visual of how training set was created. A experienced, trained, researcher was asked if a region of interest was a neuron or not.

The specific type of neural network that is used in this experiment is a convoluted neural network. This type of neural network is usually used to interpret pictures and videos. An example of a convoluted neural network is shown below.
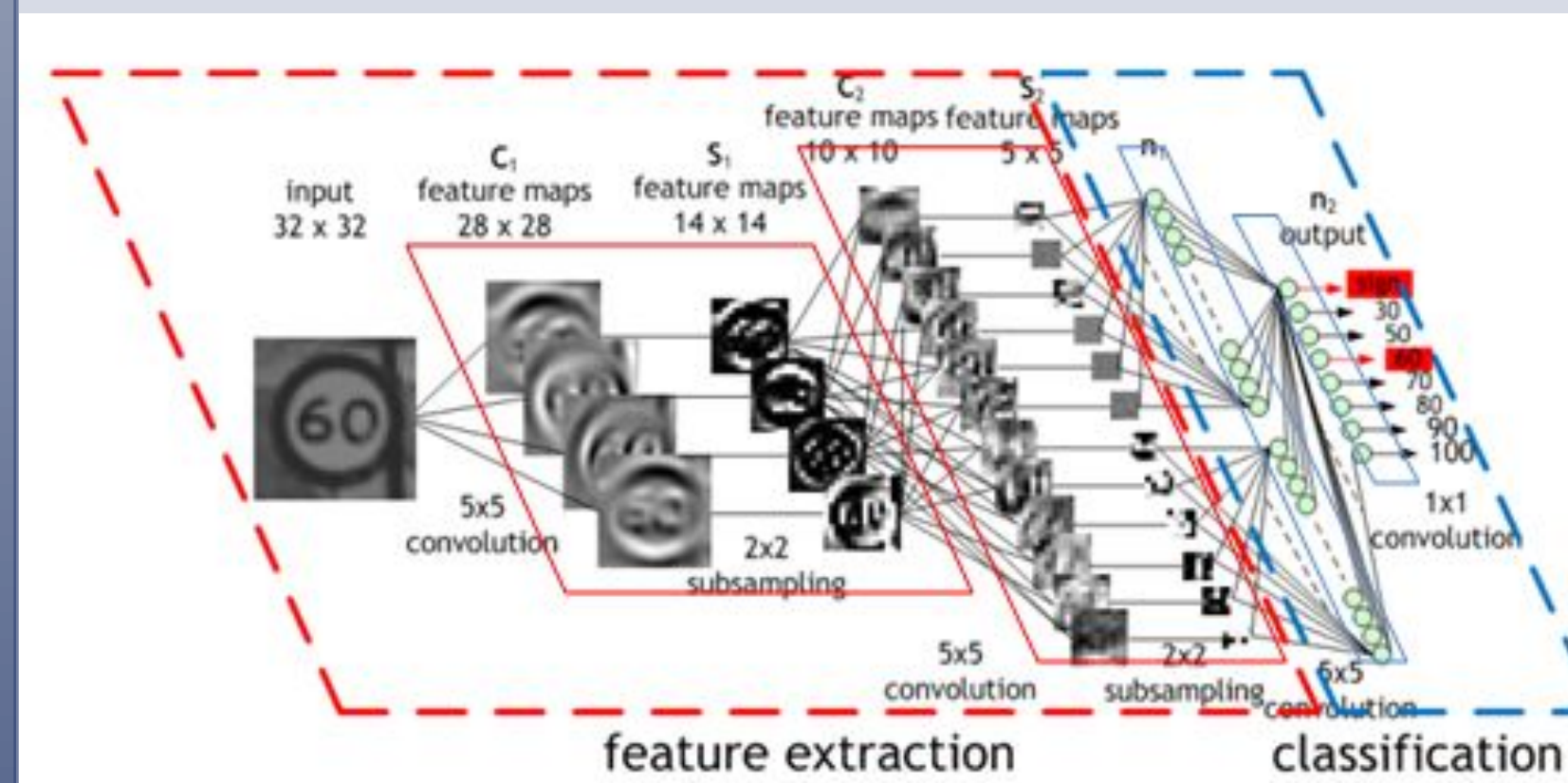

Fig. 5

Visual of how a convoluted neural network takes raw image and divides the raw data to interpret the data and return a viable output. [5]

Methods

Preparing the Mice

Mice are grown to adults, during which the genetic code for the GCaMP complex is placed into their neural cells.

Mice are then sedated, and undergo surgery to allow a small hole in their cranium for the microscope to look through.

When being imaged, mice have their heads held down steadily, although their body is more freely able to move (see below). The videos obtained are then further processed as input data for the neural network.
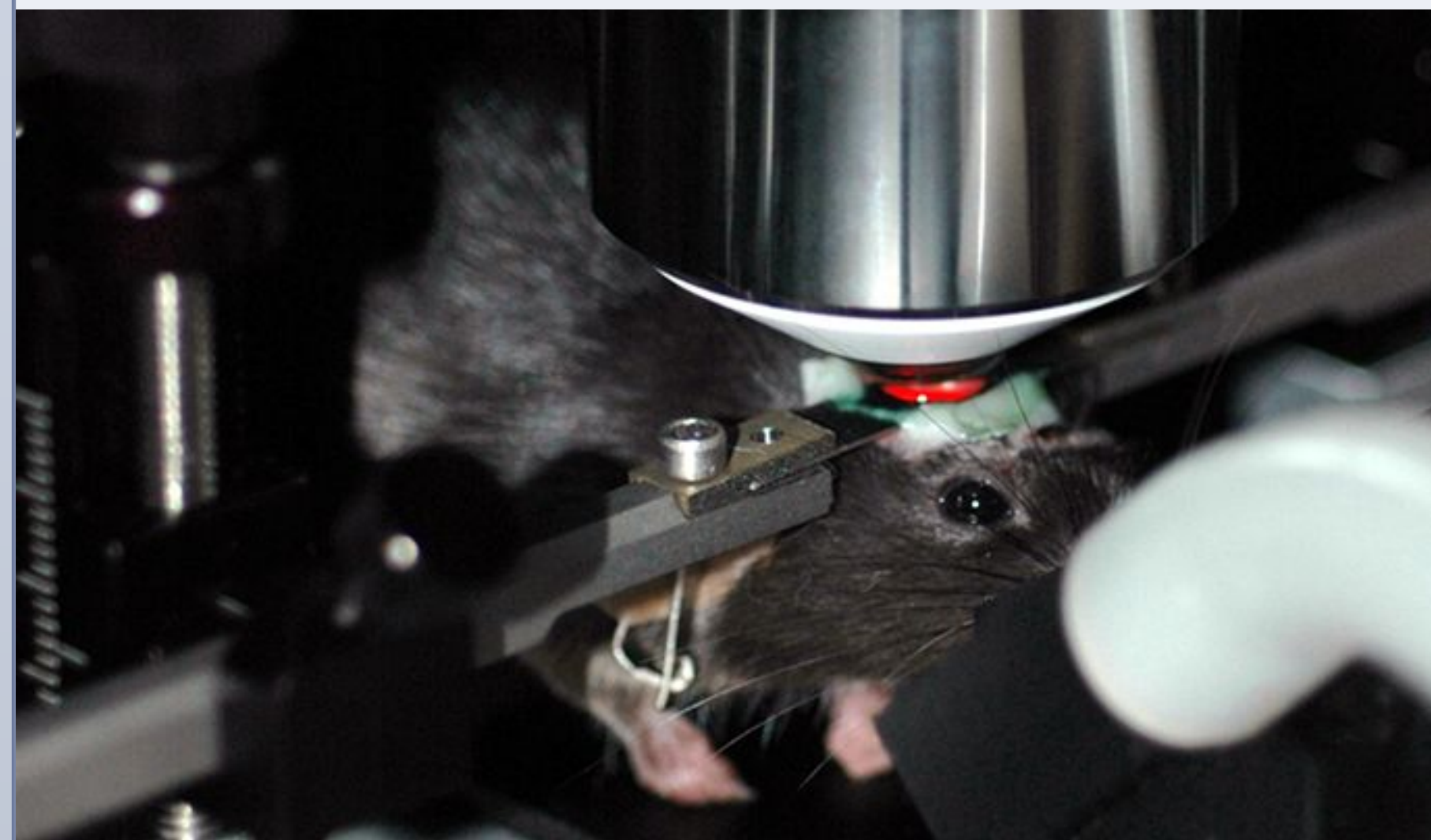

Fig. 6

Pictures of this portion of the procedure in the Gong lab could not be obtained. However, this is a example of a mouse being mounted to a microscope. Note the clamps that maintain the head and brain steady during imaging [6]

Neural Network Procedure

To input the videos taken from the mice into the neural network, the raw data must be standardized and preprocessed. Done through MATLAB, this video analysis is primarily background subtraction, although other video stabilization techniques are also used.

Next, the modified data is placed into the trained neural network and and more data is outputted

Finally, the output data is processed further to give a final map of active neurons.
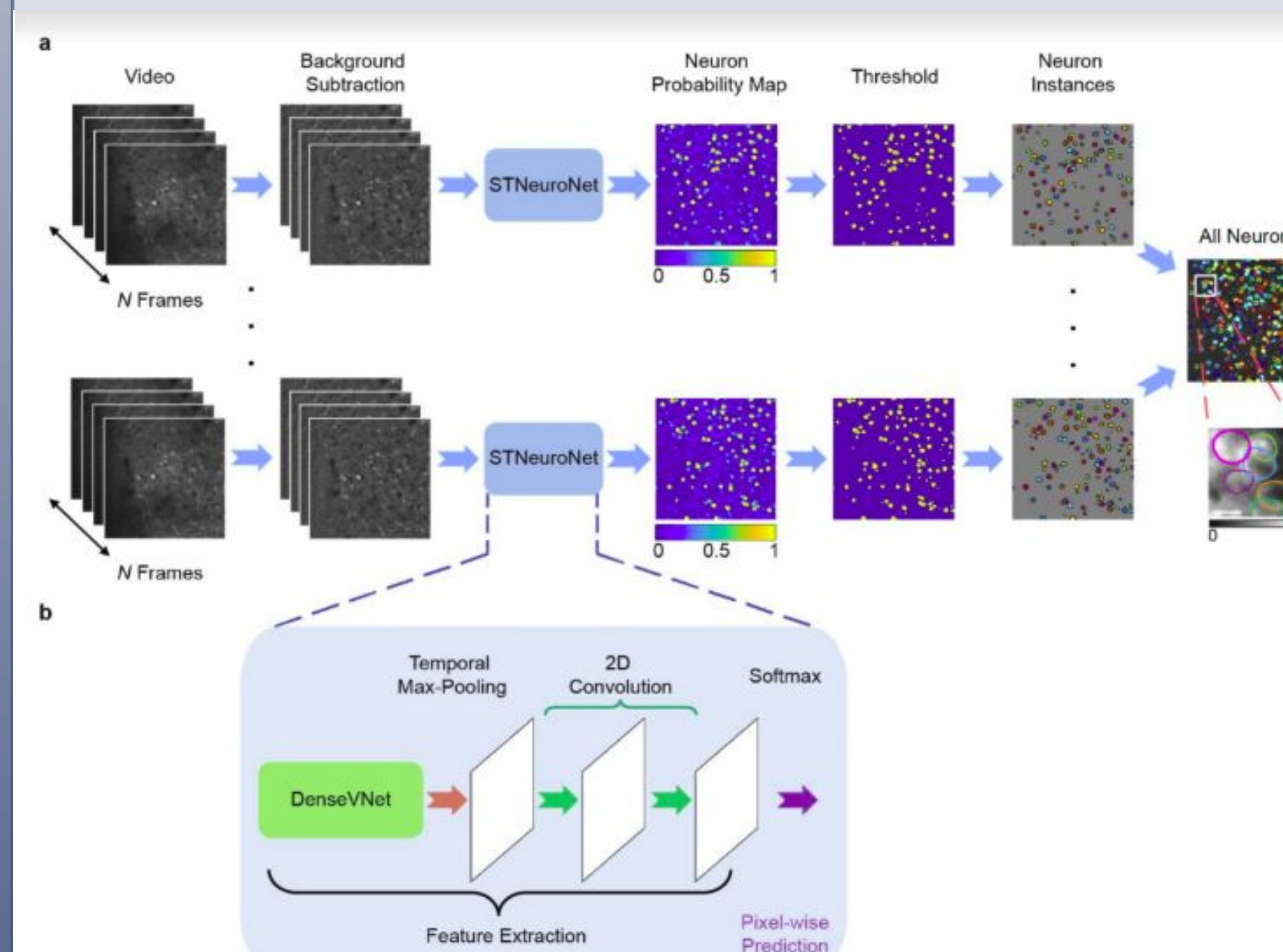

Fig. 7

Step-by-step procedure of how the raw data is preprocessed, placed into the neural network (titled "STNeuroNet") and then postprocessed. The neural network used in this project is based off of another common segmentation neural network called ""DenseVNet" with the addition of several extra computational layers.
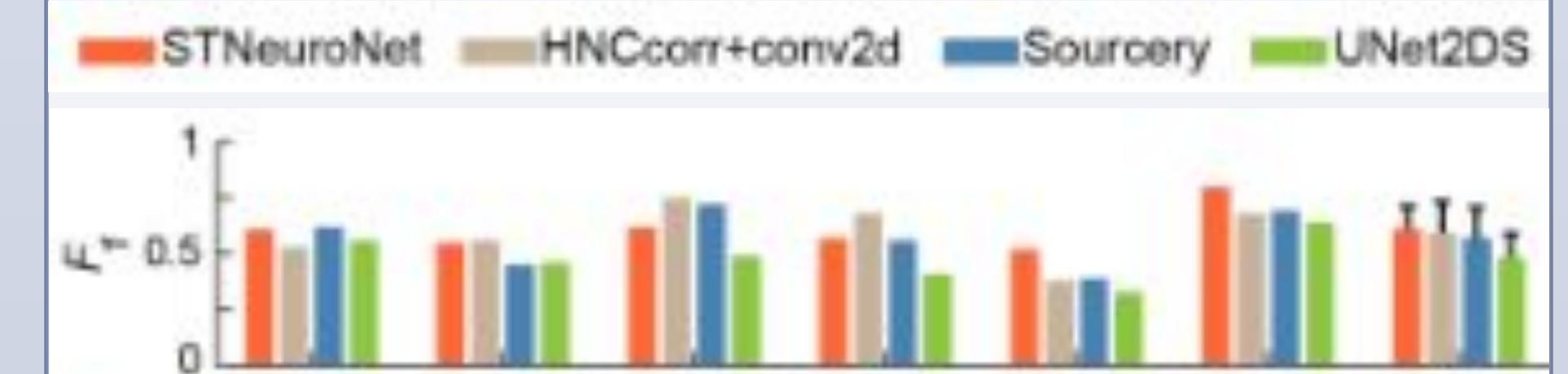
Results

Fig. 9

STNeuroNet marginally ranks first on the Neurofinder test data in comparison to other computational methods of detecting active neurons, denoted by the average F1 score across all data. Each group of bars represents a different comparison test. The F1 scores are calculated by taking the average of the precision and recall of the system. Precision is the number of correct positive results divided by the number of all relevant samples. Recall is the number of correct positive results divided by the number of all relevant samples.

Discussion

As researchers continue to uncover new knowledge about the brain everyday, our understanding of how our brain functions expands.

This project will allow for the detection and identification of active neurons *in vivo* using 1-photon microscopy. Of course, the accuracy of the program may always be improved, so future steps will include changing parameters through which STNeuroNet is developed so it will achieve a higher F1 score.

Down the road, future research may be the mapping of these active neurons and correlating them to cognitive actions. Furthermore, later scientists may also develop a better understanding of neurological diseases, as scientists would be able to see which neurons are active in diseased vs uninfected brains.

References

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