A Neural Network Based Automated IFT-20 Sensory Neuron Classifier for *Caenorhabditis elegans*

Arvind Seshan
Abstract

Determining neuronal identity in imaging data is an essential task in neuroscience, facilitating the comparison of neural activity across organisms. Cross-organism comparison, in turn, enables a wide variety of research including whole-brain analysis of functional networks and linking the activity of specific neurons to behavior or environmental stimuli. The recent development of three-dimensional, pan-neuronal imaging with single-cell resolution within *Caenorhabditis elegans* has brought neuron identification, tracking, and activity monitoring all within reach. The nematode *C. elegans* is often used as a model organism to study neuronal activity due to factors such as its transparency and well-understood nervous system. The principal barrier to high-accuracy neuron identification is that in adult *C. elegans*, the position of neuronal cell bodies is not stereotyped. Existing approaches to address this issue use genetically encoded markers as an additional identifying feature. For example, the NeuroPAL strain uses multicolored fluorescent reporters. However, this approach has limited use due to the negative effects of excessive genetic modification. In this study, I propose an alternative neuronal identification technique using only single-color fluorescent images. I designed a novel neural network based classifier that automatically labels sensory neurons using an iterative, landmark-based neuron identification process inspired by the manual annotation procedures that humans employ. This design labels sensory neurons in *C. elegans* with 91.61% accuracy.
1 Introduction

The process of neuron identification is integral to a wide range of neuroscience research. For example, it is needed to link the activity of particular neurons to specific behavior or stimuli and it enables a better understanding of the nervous system through whole-brain analysis of functional networks. Developments in imaging techniques with cellular resolution have enabled the capture of detailed neural activity within some organisms making the possibility of neuron identification, tracking, and activity monitoring enticingly close. An example of this imaging output in a Caenorhabditis elegans worm is shown in Figure 1.

The use of this imaging technology and the possibility of neuron identification is especially useful in organisms such as Caenorhabditis elegans. The C. elegans nematode is commonly used in the field of neuroscience as a model organism to study neural activity due to several unique properties it possesses. The entire C. elegans genome and connectome with only 302 neurons has been identified and well annotated. Its transparency also makes it easy to capture detailed neural activity, and its ability to self-fertilize allows for the maintaining of a genetically identical C. elegans population. [2, 7].

While new imaging technologies have helped, there remain significant hurdles in identifying neurons. The main barrier to high-accuracy neuron identification is that neuron position in adult C. elegans is not stereotyped. In fact, there is a significant level of variation in the positions of the neuronal cell bodies [12]. The ranges of possible neuron locations are visualized by the ovals in Figure 2. Current research has employed genetically encoded markers such as fluorescent proteins expressed by cell-specific promoters. This technique was recently employed for pan-neuronal classification with the NeuroPAL multicolor atlas [19]. The extensive use of genetic modification, however, inhibits worm mobility, limiting studies using the strain to short, immobilized analysis. In this study, I explore the use of other differentiating factors between neurons that could replace the use of cell markers. This results in a novel alternative neuronal identification technique that uses only single-color fluorescence, which avoids the limitations of NeuroPAL.

Figure 1: Fluorescent image of C. elegans nervous system [7].
I propose a neural network based classification system trained on annotated *C. elegans* videos to identify neurons within the worm, specifically the sensory neurons, which can help researchers correlate environmental factors to behavior through neuronal activity. I chose to use a neural network due to its flexibility and generalizability, given the high variation between worms indicated by Figure 2. In addition, recent advances of neural networks in image analysis and the ability to collect a large amount of training data makes this approach to neuron identification more practical [15]. This study aims to engineer features that aid in the differentiation of neurons in any worm, and use them to train a highly accurate neuron classification model. I train this model using three-dimensional videos of *C. elegans* being exposed to various chemicals that cause their sensory neurons to fire.

By using an iterative process that combines both location data as well as other features such as shape and fluorescent brightness, I was able to develop a neural network that correctly identified twenty-two sensory neurons with a 91.61% accuracy across six validation data sets.

The rest of the paper is organized as follows. First, I review relevant background in the field in Section 2 followed by an overview of the proposed system in Section 3 and results in Section 4. Then, a discussion of limitations and next steps is presented in Section 5 a conclusion in Section 6 and finally acknowledgments in Section 7.

### 2 Background and Related Work

In this section, I discuss the advances in imaging techniques used in this research including methods for making neurons and neuron activity visible as well as techniques for capturing three-dimensional images of organisms. I also describe current approaches for neuron identification and tracking including state-of-the-art techniques for tracking neurons over time.

#### 2.1 Fluorescent Neuron Marking

Two common marking techniques have been used to make neurons and neuronal activity visible: GCaMP calcium indicators and NeuroPAL [19].
GCaMP calcium indicators. Current is propagated in *C. elegans* neurons via calcium spikes. As a result, measuring calcium activity can be used to detect when neurons are firing, due to the predictable manner in which calcium ions exit and enter cells. Genetically encoded calcium indicators [4] have been developed to create a fluorescent glow within the neurons, glowing more brightly to indicate the neuron has fired.

**NeuroPAL.** One drawback of calcium imaging is that neuron identification is difficult because all of the neurons are the same color and both the movement of the subject and the non-stereotyped neuron position can make it difficult to use location as an identifying feature. This is especially an issue since neuron identification is typically done manually by humans, a tedious process that leads to inaccuracies if not done carefully, especially due to the significant overlap in potential neuron locations. A recent development, NeuroPAL [19], uses worms that are genetically modified with multiple colors of fluorescent reporters. The resulting images of the worm makes it possible to use a fluorescent color map to identify all neurons by color as shown in Figure 3. While promising, there are several limitations with NeuroPAL, including its toxicity to *C. elegans*, which makes it difficult to perform behavioral experiments over a long duration. This, in turn, limits NeuroPAL’s
2.2 Three-Dimensional Microscopy

A multifocal microscopy imaging system, shown in Figure 4, is employed to record the three-dimensional images with single-cell resolution used in this project. The system uses the piezo attached to the 60X objective to rapidly change the focus plane. At each focal plane, corresponding to a different depth into the specimen, the camera records a $512 \times 512$ image with a 10ms exposure time. A total of 25 different focal planes are recorded, thus, generating a $512 \times 512 \times 25$ image of the specimen every 250ms or 4 volumes/s. One drawback to this approach is the lower $z$-axis resolution (1.2µm) compared to the $x$ and $y$ resolution (0.40µm). However, this drawback is not significant for our study as there is sufficient resolution to accurately capture each neuron in all use to immobilized tests.
three dimensions. When performing NeuroPAL imaging, a 405 nm, 488 nm and 640 nm laser light sources are used while the 488 nm laser line is used for calcium activity imaging. An example of a single plane from a NeuroPAL and from a calcium image capture are shown in Figure 5 [10].

2.3 Neuron Tracking

Recent improvements in neuron tracking systems have enabled improvements in the efficiency of C. elegans analysis. One such recent tracking system is ZephIR [20]. ZephIR enables multiple object tracking within behaving animals such as the tracking of neurons within C. elegans. After manually annotating a set number of frames throughout videos, ZephIR can track objects such as the fluorescent signals within C. elegans brains over time, keeping track of which neuron is which. While this process does save time in the labeling process, there is still a significant need for human supervision in correcting incorrect frames (e.g., 15% of frames in data sets in which C. elegans is moving). However, this development enables the creation of a large labeled data set of C. elegans neurons, because manually labeled data with neuron names can be passed from one frame onto all frames in a video by tracking the neuron over time.

In this project, a ZephIR-generated data set is used to develop a neuron classification model, with the goal of eliminating the need for human involvement entirely. See Section 3.1 for more details.

2.4 Neural Correspondence

There has also been research exploring the ability to match equivalent neurons between two organisms. For example, the fast Deep Neural Correspondence (fDNC) system [21] identifies corresponding neurons between individuals with 64.1% accuracy using solely neuronal position data and 74.7% accuracy using NeuroPAL color information. Furthermore, the model is trained on semi-synthetic data, which may not be representative of the variations and noise present in real-world data. In contrast, my approach combines a broader set of position information along with other attributes to achieve significantly higher accuracy using real data.

2.5 Existing Approaches to Neuron Identification

Current neuron identification systems rely on the use of cell-specific promoters. These promoters are expressed in subsets of the neurons, allowing for greater differentiation. While practical for analyzing small subsections of the C. elegans brain, the drawbacks of the NeuroPAL strain [19] reveal its limited use for pan-neuronal analysis. Wu et al. successfully used this technique for the identification of C. elegans head neurons with 75% accuracy. They also explored the use of additional features such as fluorescent intensity and neuron shape for further discrimination of neurons [18].
3 Automated Neuron Identification System Overview

In this section, I discuss the details of the data set used in this research, the feature manipulation process, and the model architecture.

3.1 Data Set

The data set consists of sixty-five, 667.5 second long, 3D videos, each with a different *C. elegans* worms. The worms were from a population of hermaphrodites, due to their ability to self-fertilize. This means that each worm in the population is essentially genetically identical to the rest [2]. The calcium imaging process is used to measure the fluorescent glow in each neuron. Each video contains 2670 volume captures of $512 \times 512 \times 25$ pixels. Note that the calcium activity imaging used in the creation of this data set expresses a single color fluorescence. An example frame from these videos is shown in Figure 6 represented in two dimensions as three two-dimensional planes ($xy$, $xz$, and $yz$ shown on the top, bottom, and right respectively) for ease of visualization. Note that this example frame has also been cropped to where the neurons are located. This research focuses on classifying the IFT-20 sensory neurons. The intraflagellar transport (IFT) system is responsible for the formation and function of cilia. The IFT-20 subunit of the system has been found to be responsible for the function of sensory cilia [5]. These neurons are of particular importance in correlating *C. elegans* interaction with its environment to neuronal activity.

The ZephIR object tracking system [20] is used to track the neurons across time in these videos, producing coordinate locations for the neurons in each volume. The neurons are then manually labeled by their name.
3.2 Feature Set

In order to help the machine learning model better learn from the provided data, simpler features must be extracted from the data set. Our approach to generating features that would be useful to a neuron classification model is inspired by the approach that humans use to annotate the neurons manually. A common strategy for manual labeling is to identify a few specific neurons based on a combination of location, relative isolation from other neurons, brightness and shape. Once these initial few neurons are labeled, additional neurons are labeled using their location relative to the identified ones. Based on this, I chose to use five groups of features: centroid-relative coordinates, local structure, global position, shape, and fluorescent brightness. Each of these features is described in detail below.

3.2.1 Centroid-Relative Coordinates

Neuron position in 3D space is computed using the ZephIR object tracking system, which outputs coordinates for objects it determines to be neurons in the frame.

To normalize the 3D coordinate locations of each neuron, positions are expressed relative to the centroid of the neurons. Then, each component of the coordinates is divided by the average distance away from the centroid along each axis respectively. Since an average is taken, this process is robust even when some neurons are missing in particular frames. Figure 7 shows three-dimensional plots of the coordinate distribution color-coded by neuron. Note that the C. elegans brains have corresponding left and right brain neurons and that these neurons are colored the same in the figure. Figure 7a shows that there are distinct clusters of neurons even across 65 randomly selected frames where the worm stretches and moves slightly. This proves the coordinate normalization system is robust and coordinates do not change as a worm moves. Figure 7b represents the coordinate distribution for a randomly selected frame from 65 different worms. Figure 8 shows the 2D projections of this same data. The average of the standard deviations of the distances of each point to the centroid of its corresponding point cloud is 0.020 for 65 frames of the single worm and 0.238 for the single frame from 65 different worms. This metric provides an indication of how variable the positions of the neurons are across observations. There is a much larger variation in position for each neuron and larger overlap between neuron locations across the different worms, indicating the need for other features beyond centroid-relative coordinate location when identifying a large number of neurons.

Note that in addition to being a feature given to the neural network as an input, the calculation of local structure and global position below use these centroid-relative coordinates to ensure calculations such as distances are normalized.

3.2.2 Local Structure

Humans often use local structure to recognize and label similar objects. For example, it is easy to recognize and label the stars of the Big Dipper based on their relative position without knowing
their overall position in the sky or their relative brightness. For each neuron, the distances to its three closest neighboring neurons in three-dimensional space are added to the feature set to represent and convey this type of local structure information. Figure 9 shows an example of how this local structure feature is measured.

3.2.3 Global Position

The location of a neuron within some global context such as the worm’s body or worm’s overall nervous system can provide valuable information. For example, in contrast to how you recognize the Big Dipper, you can identify the North Star simply by its position in the sky, without any other information. The challenge is creating a global coordinate system to label the neuron positions in a meaningful way. Ideally, I want to give neurons position labels that minimize the variation between frames and between worms. Unfortunately, Figure 7 shows that using something simple, such as centroid-distance, to provide global position produces values that differ significantly across worms.

To generate a more robust coordinate system, I utilize a landmark based approach. The position of any neuron is defined by its distance to a number of well-known landmarks. Note that the distance to a landmark localizes the neuron to the locus of a sphere around the landmark. To pinpoint a neuron in three-dimensional space, the system requires a minimum of four landmarks, although additional landmarks can make the system more robust to measurement errors, omissions or even variation across worms.

A good landmark must be easy to find in each frame in the video and should not introduce significant variability as the worm moves or deforms. I chose to use a small, easily identifiable subset of the neurons for this purpose: ASK, ADL, and ASI. Since I can use the left and right equivalent neurons in the brain, this provides six landmark neurons (ASKL, ADLL, ASIL, ASKR,
Figure 8: 2D projections of the data shown in Figure 7 (a-c) show projections to $xy$, $xz$, and $yz$ planes, respectively, for 65 frames of a single worm. (d-f) show projections to $xy$, $xz$, and $yz$ planes, respectively, for a single frame of 65 different worms.

ADLR, and ASIR, where L and R refer to left and right brain). These neurons move along with the rest of the *C. elegans*’ brain, reducing the impact of motion or deformation as a factor. These neurons are used by manual annotators as starting reference points in neuron identification. An example of using neuron based landmarks in this way is shown in Figure 10.

A separate neural network was designed to bootstrap this process by recognizing and labeling a small number of neurons. This special neural network had all the same inputs as the general neural network other than the global position.

Training and running this simpler network identified six neurons that could be labeled with high accuracy (98%) and could serve as landmarks. See Section 3.3 for details on the architecture of this landmark neuron classifier and Section 4.2 for evaluation of the accuracy. With the identified landmarks, the location of each neuron is expressed as a vector of six values (distances to each landmark) that are passed to the neural network as features.
Figure 9: Closest three neurons to neuron marked in red are marked in blue. Yellow distances represent the local structure feature.

3.2.4 Neuron Shape and Fluorescent Brightness

The shape of the neurons as represented in the calcium imaging process differ in significant ways. For example, the AWCL neuron in Figure 11 has a more oval shape that of ASEL. To create a feature that represents the shape of the neuron, I extract the relevant pixels from the three-dimensional images of the *C. elegans* brain. These images are cropped to capture each individual neuron and parts of surrounding neurons. The dimensions of each volume crop is $19 \times 19 \times 5$. Notice the lower size in the $z$ dimension due to the lower resolution when performing multifocal microscopy. Two-dimensional representations of these volume crops can be seen in Figure 11. This crop provides information about the shape of the neuron as well as some of its immediate surroundings.

Previous studies have confirmed the ability of fluorescent intensity to differentiate neurons from their neighbors [3, 19]. While multicolor fluorescence is not provided as in NeuroPAL, single color green fluorescence could still be a helpful feature for neuron identification. The brightness values in each of these volume crops need to be normalized due to diminishing fluorescence over time caused by photo bleaching in the fluorescent proteins. To normalize the brightness, the system divides by the average brightness across the neurons in a frame.

A numeric score for the brightness of the neurons is also extracted from the fluorescent values in the volume crops. The fluorescence is averaged across the center $6 \times 6 \times 2$ pixels of the $19 \times 19 \times 5$ crop collected as part of the neuron shape.

3.3 Model Architecture

The neuron classification model consists of a combination of convolutional and fully connected layers to account for the different types of model inputs. A diagram of the neural network architecture used is shown in Figure 12.

Note that the architecture contains two instances of very similarly structured neural net-
works [1]. The top half of the diagram shows the neural network instance used to identify the landmark locations. These landmark locations are then used to compute the distances associated with our global position feature input. These distances are passed as input to the neural network in the lower half of the diagram. Below, I describe the common components of the top and bottom neural network structure.

The collection of convolutional layers of the neural network, labeled convolution1 and convolution2 in Figure 12, receives the $5 \times 19 \times 19$ volume crops and passes them through a series of 3D convolution and batch normalization layers, reducing the dimension to an output size of $5 \times 7 \times 7$. Convolutional layers are commonly used in image processing tasks [6, 9] since they capture the spatial relationship between the pixels. In contrast, processing pixels using a fully connected layer would ignore this relationship, making each output have a potential relation to every input. This makes it more difficult to train and reduces performance. The basic step in a convolutional layer is to treat the input as a matrix and convolve a second smaller matrix, called the kernel, across the input, multiplying the two matrices at each step. This reduces the input into a form that summarizes key features and is easier to process. Convolutional layers are typically used along with pooling layers [17]. Pooling layers perform a similar convolution; however, extracting the maximum or average value in the smaller matrix’s region at each step rather than multiplying. This essentially downsamples the information from the previous convolutional layers. Because of the reduction from the input to the output, the model is forced to choose what data is passed forward, essentially making it learn to only pass the useful parts of the volumes [9, 6].

There is also a collection of fully connected layers with ReLU activations [1, 11] labeled linear1 that receives the distances to the three closest neighbors associated with the local structure feature, the average fluorescent brightness value, and the centroid-relative $x$, $y$, and $z$ coordinate location.
Figure 11: 2D representations of volume crops of the ASEL (left) and AWCL (right) produced by the calcium imaging process. Note the more oval shape of AWCL.

in the image. The bottom instance of the fully connected layers labeled linear2 also receives the six landmark distances for the global position feature. As a result, the input shape of the top fully connected layers is 7 and the bottom is 13. This section creates a simple sequential model with a series of fully connected layers. Its output size is 48.

The output of the linear1 and linear2 sections are much smaller than that of their corresponding convolutional layers, potentially biasing the weights towards the volume data. Therefore, a set of fully connected layers labeled convTransform1 and convTransform2 are used to reduce the size of the convolutional layer output from $5 \times 7 \times 7$ to 48 to match the output of the linear1 and linear2 sections.

Finally, the output of the linear1 and convTransform1 are combined in as set of fully connected layers labeled output1. The final layer is a log softmax layer that produces a log probability distribution across the possible outputs. Log softmax is used as the output layer activation function because the probability distribution allows us to determine a confidence score for the predicted label. In addition, the negative log-likelihood loss function is being employed for model training. The output1 section has 7 possible neurons in the final layer, representing the 6 landmark neurons and a “not a landmark” output. The linear2 and convTransform2 outputs are processed similarly. However, the final output of the output2 stage has 22 possible neurons representing the set of IFT-20 neurons that I currently identify [1].

4 Results

To describe the performance of our neural network, I first describe the details and performance of the training process (Section 4.1). I then discuss the accuracy of the resulting model on data set aside for model validation (Section 4.2).
4.1 Model Training

There are multiple parameters that must be configured as part of the training process. First, I use the negative log-likelihood loss (NLLLoss) function for training since it is a good function for training models that are meant to classify multiple distinct classes and was empirically determined to perform best. Loss essentially describes how far predictions are from the true label. Second, I use the ADADELTA optimizer function for training. ADADELTA is a stochastic gradient descent method that has adaptive learning rates. The goal of training is to minimize the error, which is done using backpropagation, which calculates the error gradient based on model parameters. Essentially, this process provides adjustments to model parameters based on what is determined to minimize loss. Finally, to prevent overfitting, I employ early stopping using the metric of validation loss and a model weight decay of 0.001. Overfitting is when a model fits too closely to training data and thus does not generalize well and performs poorly on testing data. Early stopping prevents this by stopping the training process when the validation loss increases. Weight decay keeps weights from getting too large by penalizing complexity, essentially setting a limit to how complex the model can become.

Of the 65 data sets/C. elegans, 59 are chosen as training data and 6 for validation. As each batch is iterated over for a maximum of 256 epochs, 10 frames are selected from each. This is
Figure 13: The change in the training and validation loss during the 75 epoch training process.

because frames that are closer together tend to be more similar as worms have less time to move

Figure 13 shows the results of the training process. Notice how the early stopping system ended training early at 75 epochs when the validation loss began to rise. The training and validation losses were at 0.001 and 1.2 respectively when training ended.

4.2 Model Validation

When testing the model, I make predictions on the validation data sets by frame. This allows us to consider the fact that each neuron only occurs once in each frame, meaning that once the label has been assigned to a neuron in a frame, the possibility can be eliminated from all remaining
neurons. Mimicking the iterative assignment process during the manual annotation process, the system proceeds from the highest to lowest confidence of predictions, eliminating each label as it is assigned. This ensures that each neuron will have its own unique label within a frame. This essentially mimics a bipartite graph matching based approach between the set of neurons in the frame and the set of 22 IFT-20 neuron labels. Ensuring a one-to-one matching between predictions and labels within a frame results in a validation accuracy of 91.61% when comparing predicted neuron labels to true labels using ten frames from each of six validation data sets.

Figure 14 is a confusion matrix summarizing the performance of the system. It shows the relationship between predictions made on the validation data set and the true labels for that data. As one would expect, 91.61% of the values are along the diagonal, meaning that the true label matches the prediction.

Note three pairs of neurons show significant mislabeling with each other. AWAR is often confused with AWBR, ASER with AWAR, and ASER with AWBR. This limitation is readily explained by the anatomy of C. elegans, which produces significant neural overlap. For example, in Figure 15, I can see that the regions that mark the potential location of AWAR and AWBR, overlap significantly. Similarly, the other two aforementioned pairs have overlapping coordinate distributions. Even when manually labeling such neurons, confusion between these neurons is common.
5 Discussion

While the above results show that the system performs extremely well at labeling neurons, there are a few potential areas for improvement. Below, I describe three possible enhancements to the system.

Pan-neuronal Classification. The most significant next step is to scale the model to a pan-neuronal classification system. The detailed feature selection process in this research likely will generalize well to the entire C. elegans brain, serving as a complete replacement for the NeuroPAL \cite{19} color atlas. Using a NeuroPAL strain of C. elegans, a data set can be created for a model that predicts a color output. That way, manually labeling the neurons, which would be a tedious process for pan-neuronal data, is unnecessary. A model would, instead, produce a continuous output in a color space, essentially creating an artificial NeuroPAL representation.

Confused-Pair Specific Models. As noted previously, overlapping neurons is a concern in C. elegans whether manually or automatically labeling them. Since neuron location was found to be the most influential feature in determining neuronal identity, the model faced difficulty in discriminating between neurons such AWAR and AWBR, whose coordinate distributions have significant overlap. This drawback could potentially be overcome by forming several binary classification models designed specifically for neuron pairs that are often confused, allowing for specialized weighting on other features such as shape and fluorescent brightness.

Cross-frame Classification. In addition, while the current feature set (Section 3.2) is inspired by existing approaches to manual labeling, there is one source of information that humans commonly use that our system does not leverage: cross-frame tracking. As a worm moves and reacts to the environment, different neurons are activated. In some frames of captured data, the specific neurons are much easier to accurately label. From a particular labeled image, tracking mechanisms can be
leverage to improve the labeling of both frames before and after the labeled one. One approach to creating a system of this type is to use a recurrent neural network, where the last prediction of neuron locations is kept as hidden internal state to aid in generating the subsequent predictions. To improve the labeling of earlier frames, the video can be processed in both forward and reverse since tracking mechanisms across frames are likely to be robust to the direction of playback. In essence, this approach combines the strength of the system described in this paper with previous efforts such as ZephIR [20].

Finally, I hope to try to interpret the result of applying machine learning to neuron identification to see if there is any intuition that can be used to aid other manual and automated approaches to neuron identification. This may require using alternate machine learning approaches that lend themselves better to human understanding.

6 Conclusion

This study shows that it is possible to develop an accurate and automated method to identify neurons in *C. elegans*. By using an iterative process that is landmark based, I am able to consistently identify neurons, far more easily than the manual methods used today. The neural network I designed is able to achieve an accuracy of 91.61% for classifying twenty-two sensory neurons in six validation data sets. This high-accuracy, automated neuron identification system has the potential to aid in research studying the correlation between external stimulants, such as the introduction of a chemical substance, and *C. elegans* behavior.

As a key step towards the creation of a pan-neuronal classifier, this research is fundamental to enabling the analysis of whole-brain activity and functional networks. In particular, capturing a complete view with circuit-level detail is impossible without neuronal identification [19]. NeuroPAL enables this analysis for essentially immobilized *C. elegans* and the research presented in this paper has the exciting potential to provide the benefits of NeuroPAL for freely behaving animals.

In addition, this system opens up a vast range of applications in the study of not only *C. elegans* but also other organisms where manual labeling of neurons has been a significant limitation. For example, it could be adapted to enable neuron identification in other organisms such as the zebrafish, which has recently become of much interest to neuroscientists. At the larval stage, these fish have 100,000 neurons and are transparent, making them ideal subjects for the study of neuronal activity [16].

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