

Detection of Neurons in the Proteomic and Genomic Image Data



Aswin Kumer S V, Pamarthi Kanakaraja, Pathan Bahalul Khan, Vudatha Veera Venkata Dileep, Challapalli Ujwala

Abstract: Nowadays, the major biomedical data required for diagnosing the disease is neurons in the nerve cell. Just a brief timeframe after the neuron became recognized as the basic unit of the sensory system, the main endeavors were made to appraise the quantity of neurons in various parts of the sensory system. During the previous century, an incredible number of techniques have been utilized in making such gauges. In spite of the fact that the most generally utilized and acknowledged strategy is that of direct including in the magnifying lens, different systems, including photographic, projection, homogenate, programmed, and visual strategies have been planned. And in this project we are taking a brain tissue as an image data and from that image we are finding the number of neurons which are active in state for the first 24 hrs. and again check for 48 hrs. and finally for 72 hrs. so we here find how neurons are responding after giving information to a body and that information flows through nerves of the body and reaches to the neurons present in a human brain and the neurons react to the information and we take the data that how many neurons are responding to the information that is given to a human body. So, by finding the number of neurons responding to the information given to human body we could estimate the neurons which are alive, and which are dead by this we could declare the mental status of a person. So we are finding the number of neurons with the help of neural network method using MATLAB software and we created a page with the help of MATLAB so we can give input image in the page and the code we written will help to check the number of neurons.

Index Terms: Neural Networks, Machine Learning, Image Segmentation, Image Counter.

I. INTRODUCTION

An important problem in contemporary neurobiology is the accurate classification and counting of cells in brain structures.

Several researchers have found a correlation between neuron number and brain function. Subtle variation in the density of neurons has been shown to have a profound effect on behavior.

Neuron number has also been shown to be a good measure of the severity of disease. Cotman and Anderson, for example, have suggested that there appears to be a correlation between programmed neuron death and Alzheimer's disease[1]. Additionally, systematic changes in neuron number in certain brain structures appear to correspond to normal developmental processes.

An ideal neuron counting system would obtain an accurate neuron count in a sample in a small amount of time. Due to the large number of neurons in a brain sample, manually counting every neuron is unrealistic. Many researchers have worked on the problem of estimating the number of neurons in a tissue sample by counting neurons in small regions and applying statistical models to extrapolate the neuron population in the entire sample. Abercrombie developed an early method for estimating neuron count. His algorithm, however, was biased and tended to result in falsely high estimates. Sterio introduced an unbiased neuron estimation method which has proven popular. Several other researchers have also developed unbiased models for estimating neuron count. Unfortunately, more accurate estimates of neuron count require computationally more expensive estimation models.

The number of neurons within a sample is typically obtained by performing a histological analysis of some regions of the sample. This set of counts is then used to estimate total neuron count within the sample. Consider, for example, Fig. 1 which represents a tissue sample. The spherical regions represent neuronal nuclei within the sample. In order to obtain a clear image of individual neurons the thickness of the tissue sample must be on the order of the size of a neuron[1]. Thicker samples are analysed by slicing the sample into several sections. Possible slice positions are illustrated in Fig. 1 by dashed lines.

Immunostaining is the process by which the neurons or neuronal nuclei are chemically treated such that their spectral transmission is different from that of the surrounding tissue. In counting applications, nuclei are often preferred to entire neurons because they are nearly spherical and are usually well separated. Several staining processes are commonly used including diaminobenzidine (to produce a brownish-red colour) SK peroxidase (to produce a blue-grey colour), alkaline phosphatase, and peroxidase coupled dyes. The staining process used in a particular experiment is the one which maximizes neuron discriminability. In some experiments multiple classes of cells can be discriminated by treating the sample with more than one stain.

Manuscript published on November 30, 2019.

* Correspondence Author

Aswin Kumer S V*, Department of Electronics and Communication Engineering, Koneru Lakshmaiah Education Foundation, Vaddeswaram, A.P., India.

Pamarthi Kanakaraja, Department of Electronics and Communication Engineering, Koneru Lakshmaiah Education Foundation, Vaddeswaram, A.P., India.

Pathan Bahalul Khan, Department of Electronics and Communication Engineering, Koneru Lakshmaiah Education Foundation, Vaddeswaram, A.P., India.

Vudatha Veera Venkata Dileep, Department of Electronics and Communication Engineering, Koneru Lakshmaiah Education Foundation, Vaddeswaram, A.P., India.

Challapalli Ujwala, Department of Electronics and Communication Engineering, Koneru Lakshmaiah Education Foundation, Vaddeswaram, A.P., India.

© The Authors. Published by Blue Eyes Intelligence Engineering and Sciences Publication (BEIESP). This is an [open access](http://creativecommons.org/licenses/by-nc-nd/4.0/) article under the CC-BY-NC-ND license <http://creativecommons.org/licenses/by-nc-nd/4.0/>.

Detection of Neurons in the Proteomic and Genomic image Data

The section shown in Fig. 2, for example, has been stained using a terminal deoxynucleotidyl transferase (TdT) labelling technique which causes damaged nuclei to appear black. This section has also been treated using a Bcl-2 labelling technique which appears as a brownish coloration of the neuronal tissue as well as the grey appearance of the glial cells.

After staining, individual sections appear as in Fig. 2 and are ready for counting. Each section is examined under a microscope and regions corresponding to neurons, which are referred to as neuron profiles, are classified and catalogued. Neuron profile counts from each section are then combined by various methods to estimate total neuron population within the sample. The optimum method to estimate neuron count from neuron profile count is a subject of debate in the neurobiological community[2]. The debate centre's on the error introduced during the sectioning process. Due to their finite size, individual neurons are commonly cut into more than one section. This results in single neurons creating multiple profiles. Most currently employed techniques for estimating neuron count make use of statistical properties of the distribution of neurons throughout the sample. A more accurate method of estimating cell count would be to reconstruct the volume, thereby merging overlapping neuron profiles in adjacent sections into single neurons.

The system makes use of conventional neurobiological methods for neuron staining and sample sectioning. The system replaces the labor intensive manual classification and counting step with a machine vision module. The machine vision module makes use of a Machine learning defined on the colour space of the imaging system and subsequent geometric analysis to isolate neuron profiles within sections. Rather than estimating total neuron count from neuron profile counts in a set of sections, the system reconstructs the volume from the neuron profiles by combining overlapping profiles in adjacent sections into single neurons. The system thus provides an accurate neuron count within a tissue sample[3].



Fig. 1: Brain Tissue Sample

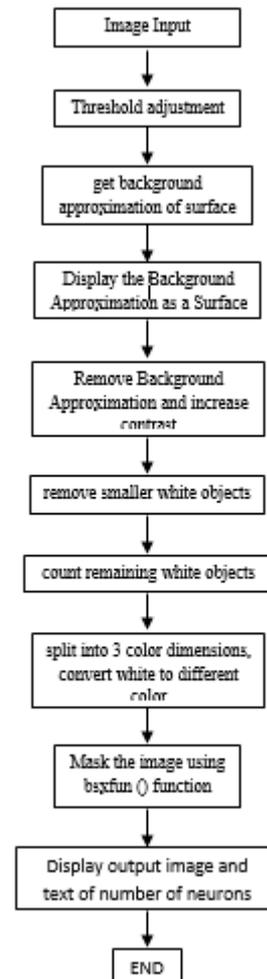
II. RELATED WORK

The brain tissues are scanned by many various methods with the help of our 20th century equipment like EEG, PET (positron emission tomography) and this brain tissue is scanned at different times in a day and keep the data as image and the machine learning panel is created with a neural network code written as background work done in the panel and we give the image data and set the threshold value

as the size of neuron and the code is used find the active stage neurons from the tissue data we given as input and this is all done by the code written with the help of neural network. Finally, we get the number of neurons which are active so that we could say the number of neurons are alive in the tissue that are active and the other neurons are treated as dead neurons and we could find the damaged neurons from which are alive.[4]

III. PROPOSED METHODOLOGY

The initial step of tallying of neurons is the picture securing. Here, it can be done by scanning the brain using EEG (Electroencephalography) and get the sample image of brain tissue and from there onwards we take three images of same brain tissue at different timings with a gap of maximum of 24hrs and we created a machine learning page with the help of neural network code in MATLAB software and we give the first image of the brain tissue as input and that will find the number of neurons that are responded for the information give to the human body so that we could come to a conclusion that number of neurons are in alive state and the other neurons are dead in that brain and by the number of neurons.



IV. CELL LABELING

In this section, we define the method employed to recognize each instance of each kind of cell in a color image.

A color imaging system obtains three measurements at each image location (x, y) given by

$$I_i(x, y) = \int l(\lambda)t(x, y, \lambda)f_i(\lambda)d\lambda \quad 1 \leq i \leq 3 \quad (1)$$

where λ denotes wavelength, $l(\lambda)$ is the spectral distribution of the illumination, $t(x, y, \lambda)$ is the total spectral transmittance of the sample, and $f_i(\lambda)$ is the sensitivity of the i th sensor class. For a red, green, blue color imaging system, for example, $f_1(\lambda)$, $f_2(\lambda)$, and $f_3(\lambda)$ are the sensitivities of the red, green, and blue sensing elements.

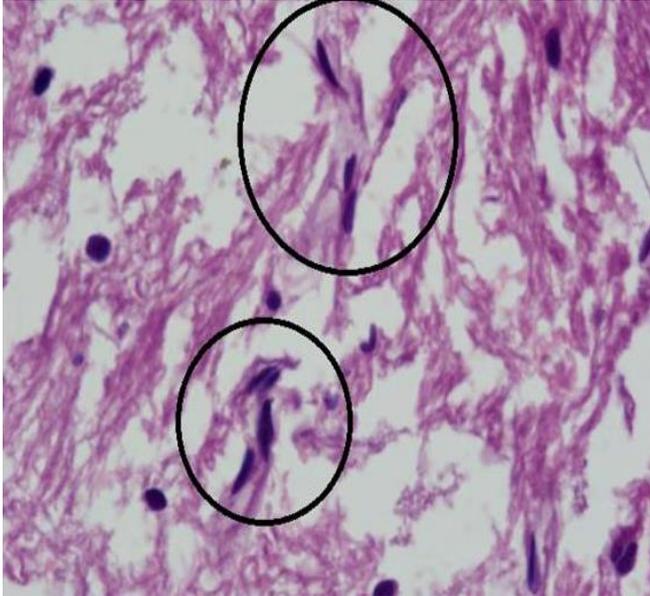


Fig. 2: Multiple class discriminability

V. PIXEL CLASSIFICATION

Let c_j refer to a class of image objects corresponding to a distinct kind of cell stained using a specific process. Assume that there is 'n' such classes C_1, C_2, \dots, C_n . Let C_0 refer to the background class. A distribution of color pixel values is observed in the image corresponding to each class c_j [5,7]. Variability in color for a single class is due to many factors including cell thickness, spatial variation in spectral transmittance, and sensor noise. Let $p(I/c_j)$ refer to the probability density of color pixel measurements for class c_j where $I = (I_1, I_2, I_3)$ is the color pixel measurement vector. Let $p(c_j)$ be the a priori probability that a pixel in each image is from class c_j . Discriminant functions for a minimum error rate Machine learning are defined by

$$g_j(I) = p\left(\frac{I}{c_j}\right)p(c_j) \quad 0 \leq j \leq n \quad (2)$$

The classifier proceeds by assigning each image measurement vector $I(x, y)$ to class c_j if $g_j(I) > g_i(I)$ for all $i \neq j$. Thus, the discriminant functions in (2) associate with each color pixel measurement the most likely class [6]. Ideally, the probability densities $p(I/c_j)$ do not overlap meaning that for any color I at most one of the functions $p(I/c_j)$ ($0 \leq j \leq n$) will be nonzero. This means that any observable color I corresponds to a unique class and pixel classification will proceed with zero incorrectly classified points. If there is significant overlap among the probability densities, then a single color in the image can correspond to

more than one class. A staining scheme that leads to significant overlap would not be useful for cell classification using color information.

The probability densities $p(I/c_j)$ are estimated from representative labeled image data of class c_j . The a 'priori' probabilities are estimated similarly from labeled images or can be assigned using expert knowledge about the expected density and size of cells of a kind in the brain area under analysis.

If the value of $g_i(I)$ is small for all classes for a color pixel measurement I during pixel classification, then such a measurement is unlikely given the modeled classes. If many such pixels are encountered during pixel classification, this suggests that some aspect of the staining and imaging process is inconsistent with the prototype processes used during estimation of the class densities $p(I/c_j)$ [6,7]. Such images are rejected by the system before being incorporated into the database.

VI. GEOMETRIC ANALYSIS

The result of pixel classification is to classify each pixel in the image into one of the classes C_0, C_1, \dots, C_n . Following pixel classification, it is possible for a few outlier background and cell points to receive an incorrect classification. These points are eliminated by morphological operators that remove components that are smaller than a minimum cell size and fill in missing points within each cell with the dominant cell label [8]. A connected components analysis on each class isolates the connected image regions, corresponding to each class of stained cell and background.

At this point, overlapping cells of the same class in the image are labeled as a single region. Further geometric analysis separates these regions into discrete cells. The cells appear in images as ellipses which are convex shapes. Regions formed by overlapping cells, however, give rise to strong concavities as illustrated in Fig. 3. The system analyzes each connected component for concavities by boundary tracking and at the same time fits an ellipse to the component. Region boundaries with strong concavities that are not well fit by ellipses are segmented at tangent discontinuities. The process continues the segmented boundaries until convex elliptical regions are found.

Following geometric analysis, each cell in the image has been located and classified. At this point, the number of cells in each class is computed as well as size and spatial distribution statistics [6].

VII. DATABASE MANAGEMENT

The neuron counting and classification system is embedded within an object-oriented database management system. The role of the database management system is to provide an efficient means of cataloging many neuron classes and to provide an intuitive user interface to the cataloging and classification system.

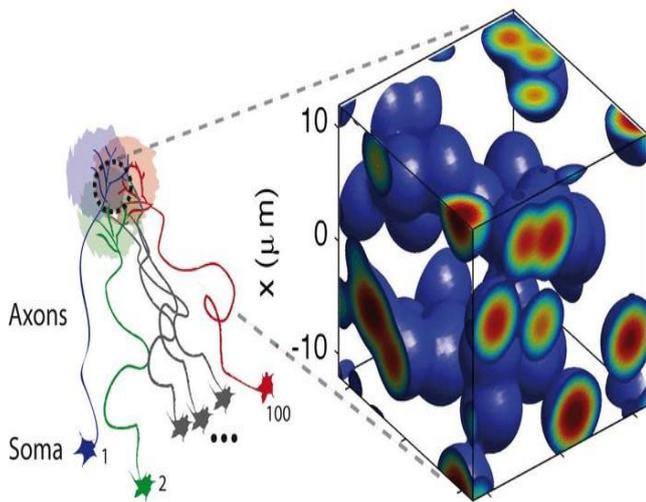


Fig. 3: Overlapping neurons

A complete brain contains several special regions which in turn contain many cells, making direct cell storage prohibitive. Many cell classes share properties however, making cataloging amenable to object-oriented database technology [9]. The database engine is optimized to perform efficient indexing in several relevant contexts. The database management system can catalog all cell classes under consideration and provide convenient handles to the Machine learning discriminant functions.

VIII. VOLUME RECONSTRUCTION

In section 2, we described a robust methodology for classifying and counting cells within an individual section. We now describe in more detail a method that can be used to obtain accurate cell counts for volumes containing several sections. The challenge lies in the possibility that a single cell can be divided by sectioning and appear in more than one section. To obtain accurate estimates of cell counts, these cells should only be counted once.

Accurate cell counts for a volume are obtained by partitioning the volume into adjacent sections. Cells in each section are identified using the Machine learning scheme and geometric analysis described in section 2. Images taken from adjacent sections are registered using fiducial points in the images. Identified cells in adjacent sections at the same location in three dimensions are interpreted as different parts of the same cell [6,8]. This allows a three-dimensional reconstruction of the cells in the volume as well as accurate cell counts. This process will be demonstrated in section 7.

IX. EXPERIMENTAL RESULTS

To demonstrate the performance of the automated neuron counting system, we present a series of images processed by the system. Images were obtained using an RGB color camera and a 24-bit RGB frame-grabber. The camera was mounted to an Olympus BH-2 microscope with a x10 DPlan objective (N.A. = 0.17) and an NFK 2.5 XLD photoeyepiece. Neutral density filters were used to ensure that the captured images used the full dynamic range of the camera/frame-grabber system without saturation.

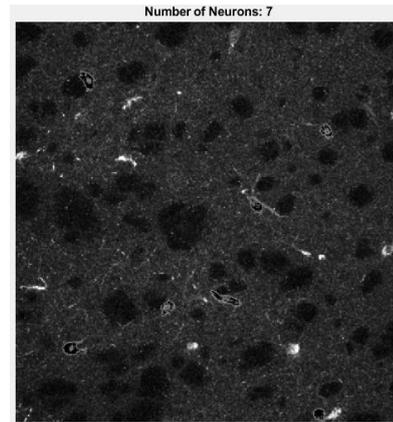


Fig. 4: after 24Hr observation no of neurons is connected state in diff type of vision

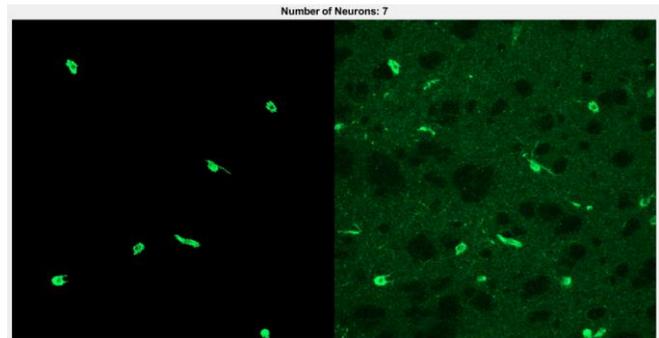


Fig. 5: after 24Hr observation no of neurons is connected state in montage type of vision

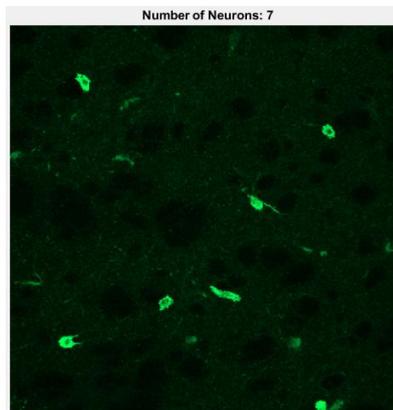


Fig. 6: after 24Hr observation no of neurons is connected state in blend type of vision

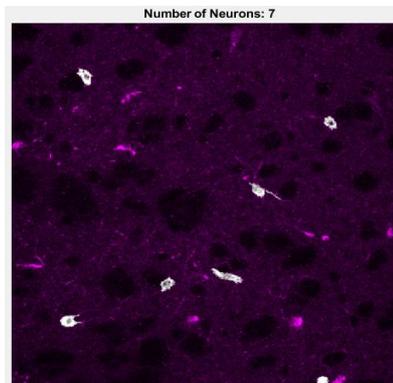


Fig. 7: after 24Hr observation no of neurons is connected state in false color type of vision

Detection of Neurons in the Proteomic and Genomic image Data

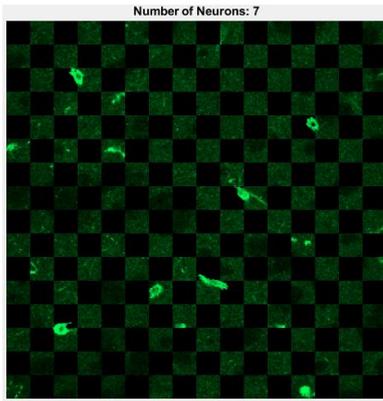


Fig. 8: after 24Hr observation no of neurons is connected state in checkboard type of vision

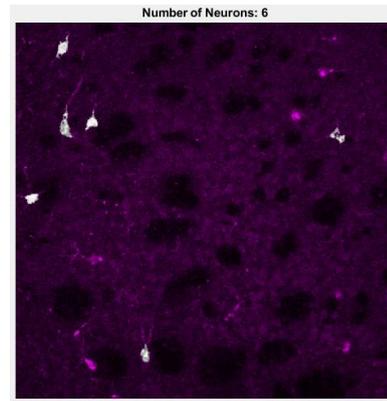


Fig. 12: after 48Hr observation no of neurons is connected state in false color type of vision

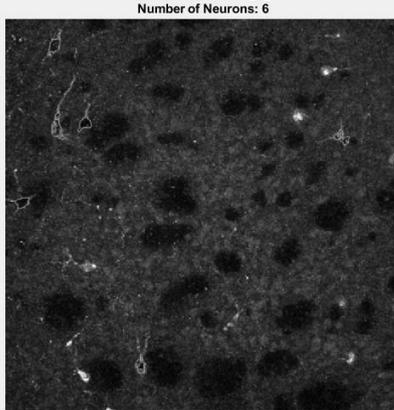


Fig. 9: after 48Hr observation no of neurons is connected state in diff type of vision

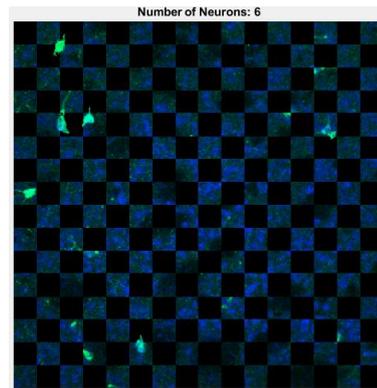


Fig. 13: after 48Hr observation no of neurons is connected state in checkboard type of vision

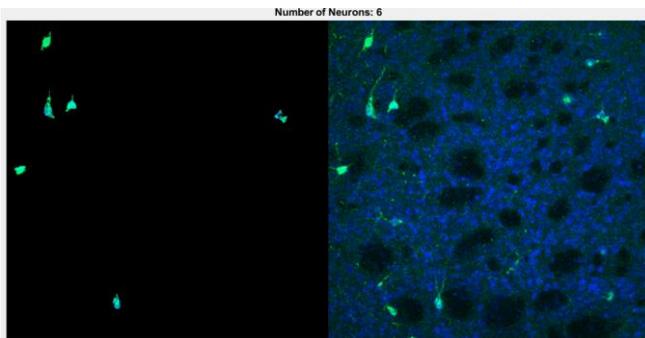


Fig. 10: after 48Hr observation no of neurons is connected state in montage type of vision

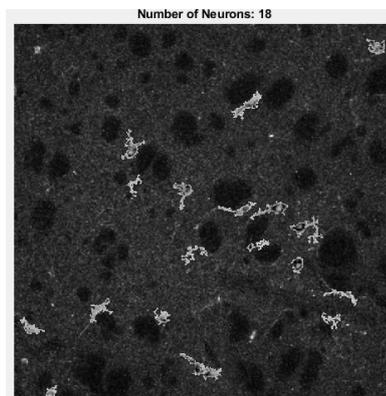


Fig. 14: after 72Hr observation no of neurons is connected state in diff type of vision

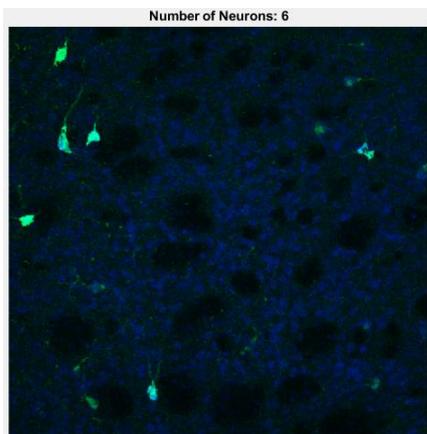


Fig. 11: after 48Hr observation no of neurons is connected state in blend type of vision

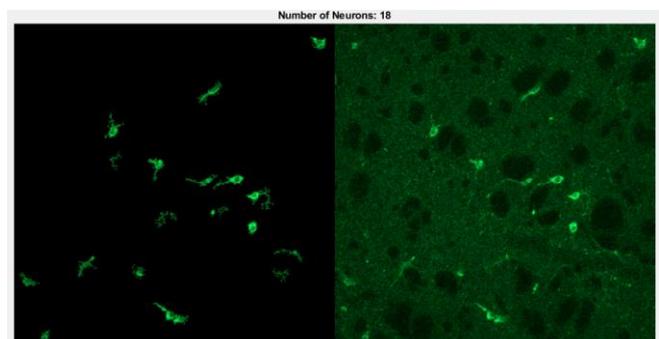


Fig. 15: after 72Hr observation no of neurons is connected state in montage type of vision

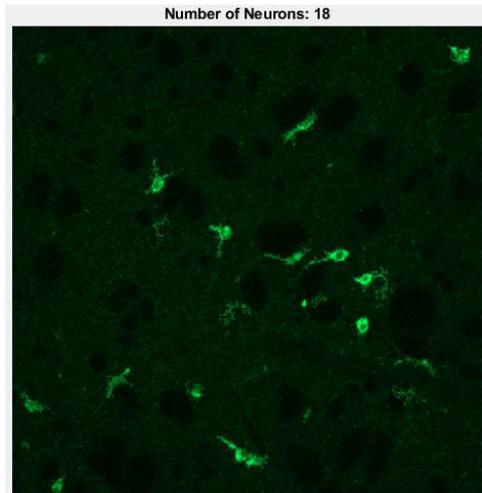


Fig. 16: after 72Hr observation no of neurons is connected state in blend type of vision

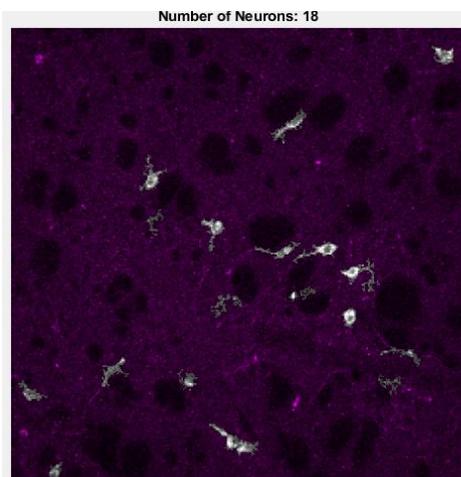


Fig. 17: after 72Hr observation no of neurons is connected state in false color type of vision

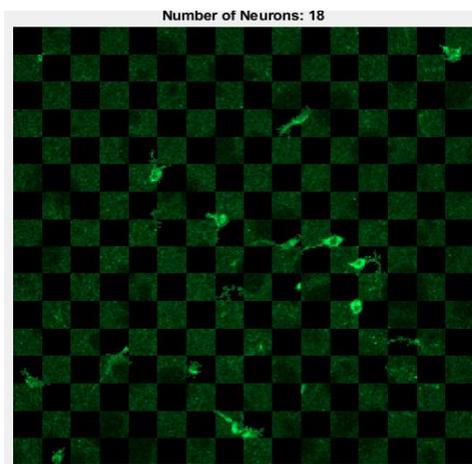


Fig. 18: after 72Hr observation no of neurons is connected state in checkboard type of vision

The Extension of this paper can be done by finding either the neuron dead or alive and if it is alive, we should find the damage percentage of the neuron this is the extension we could add to this paper

X. CONCLUSION

We have developed an automated machine vision neuron counting system. Current neurobiological technology requires a skilled technician to count neuron profiles manually in brain tissue samples and then estimate neuron

count from neuron profile count. Manual counting is tedious and prone to errors. The automated system uses a Machine Learning to discriminate neurons from surrounding tissue. The system reconstructs the tissue sample from its sections and combines neuron profiles which overlap in adjacent sections, thereby achieving an accurate neuron count. The performance of the system has been demonstrated on several tissue samples.

REFERENCES

1. M. Abercrombie. Estimation of nuclear populations from microtome sections. *The Anatomical Record*, 94:239-247, 1946.
2. S. T. Bok and M. J. Van Erp TaaIman Kip. The size of the body and the size and the number of nerve cells in the cerebral cortex. *Acta Neurological Morphology*, 3:1-22, 1939.
3. C. W. Cotman and A. J. Anderson. A Potential role for apoptosis in neurodegeneration and Alzheimer's disease. *Molecular neurobiology*, 10:19-32, 1995
4. Aswin Kumer S V and Dr. S.K.Srivatsa, "A novel image fusion approach using high resolution image enhancement technique", *International Journal of Pure and Applied Mathematics* Vol.116, No. 23, 2017, pp.671 – 683, ISSN: 1311-8080 (printed version); ISSN: 1314-3395 (on-line version), Special Issue.
5. Aswin Kumer S V and Dr. S.K. Srivatsa, "An Implementation of Suitable Image Fusion Approaches Based on the Various Input Images" *International Journal of Engineering Science and Technology*, Vol.9 No.12, Dec 2017, ISSN:0975-5462, pp.1033-1037.
6. Aswin Kumer S V and Dr. S.K. Srivatsa, "An image fusion technique based on Sparse Wavelet Transform and Non singleton Type-2 FNN Techniques", *TAGA Journal of Graphic Technology*, Vol.14, 2018, ISSN:1748-0345, pp.76-86.
7. Aswin Kumer S V and Dr. S.K. Srivatsa, "An Implementation of Futuristic Deep Learning Neural Network in Satellite Images for Hybrid Image Fusion" *International Journal of Recent Technology and Engineering (IJRTE)*, Volume-8, Issue-1, May 2019, ISSN: 2277-3878, pp.484-487.
8. Inthiyaz, S., Madhav, B.T.P. & Kishore, P.V.V. 2018, "Flower image segmentation with PCA fused coloured covariance and Gabor texture features based level sets", *Ain Shams Engineering Journal*, vol. 9, no. 4, pp. 3277-3291.
9. Inthiyaz, S., Madhav, B.T.P. & Madhav, P.V.V. 2017, "Flower segmentation with level sets evolution controlled by colour, texture and shape features", *Cogent Engineering*, vol. 4, no. 1.
10. Inthiyaz, S., Madhav, B.T.P., Kishore Kumar, P.V.V., Vamsi Krishna, M., Sri Sai Ram Kumar, M., Srikanth, K. & Arun Teja, B. 2016, "Flower image segmentation: A comparison between watershed, marker controlled watershed, and watershed edge wavelet fusion", *ARNP Journal of Engineering and Applied Sciences*, vol. 11, no. 15, pp. 9382-9387.
11. Katta, S., Siva Ganga Prasad, M. & Madhav, B.T.P. 2018, "Teaching learning-based algorithm for calculating optimal values of sensing error probability, throughput and blocking probability in cognitive radio", *International Journal of Engineering and Technology (IAE)*, vol. 7, no. 2, pp. 52-55.
12. Tripathi, D.P., Pardhasaradhi, P. & Madhav, B.T.P. 2018, "Statistical parameters-based image enhancement techniques in pure and Nano dispersed 6O.O8 liquid crystalline compounds", *Phase Transitions*, vol. 91, no. 8, pp. 821-832.
13. Madhav, B.T.P., Pardhasaradhi, P., Kishore, P.V.V., Manepalli, R.K.N.R. & Pisipati, V.G.K.M. 2016, "Image enhancement of nano-dispersed N-(p-n-decyloxybenzylidene)-p-n-hexyloxy aniline using combined unsharp masking", *Liquid Crystals Today*, vol. 25, no. 4, pp. 74-80.
14. Rambabu, M., Prasad, K.R.S., Madhav, B.T.P., Venu Gopalarao, M., Pardhasaradhi, P. & Pisipati, V.G.K.M. 2016, "Determination of phase transitions in hydrogen bonded complexes (NOBA: PFOA) using textural image processing techniques", *ARNP Journal of Engineering and Applied Sciences*, vol. 11, no. 1, pp. 520-527.
15. Sivaram, K., Rao, M.C., Giridhar, G., Tejaswi, M., Madhav, B.T.P., Pisipati, V.G.K.M. & Manepalli, R.K.N.R. 2017, "Synthesis and characterization of thiol - Capped silver nanoparticles and their effect on liquid crystals", *Rasayan Journal of Chemistry*, vol. 10, no. 1, pp. 16-24.

AUTHORS PROFILE



Dr. Aswin Kumer S V graduated in Electronics and Communication Engineering from Pallavan College of Engineering, Kanchipuram in April 2008 and received his Master's degree in Embedded System Technology SRM University, Kanchipuram in May 2012. He received his doctoral degree for the implementation of image fusion using Artificial Neural Network from SCSVMV (Deemed to be University), Enathur in February 2019. He is working as an Associate Professor in Department of Electronics and Communication Engineering at KLEF (Deemed to be University), Guntur. He has more than 11 years of teaching experience. His areas of interest are Digital Communication and Digital Signal Processing.



Pamarthi Kanakaraja is currently working as an Assistant Professor in KLEF (Deemed to be University). He has 8 years of working experience On Embedded Designing & Programming Concepts. He is a Technical EMBEDDED DESIGNING concepts Adviser for many Engineering and Polytechnic (DIPLOMA) Students. He also published papers in various international journals. He is a Regular Contributor in EFY (Electronics for You) International Technical magazine. His area of research is Embedded in Designing, Internet of Things (IoT) & Artificial Intelligence (AI). He is now doing research in radio frequency and microwave engineering. He has done many projects based on IOT and embedded systems.



Pathan Bahalul Khan, Under graduate Student, Department of Electronics and Communication Engineering, Koneru Lakshmaiah Education Foundation (Deemed to Be University), Vaddeswaram, A.P., India. His area of Interests are VLSI technology, Machine Learning, Embedded in Designing and (IoT) Internet of Things. He has done some projects in digital systems and digital communications. One of the major project he has done is Automatic Irrigation System on Sensing Soil Moisture Content which is very useful in real time farming applications. He has done certification in Introduction to FPGA Design for Embedded Systems offered by coursera. He has done various academic projects related to the embedded systems and digital electronics.



Vudatha Veera Venkata Dileep, Department of Electronics and Communication Engineering, Koneru Lakshmaiah Education Foundation, Vaddeswaram, A.P., India. Area of Interests in VLSI, Machine Learning, Embedded in Designing (IoT) Internet of Things. I have done a few hardware projects in my core domain and has basic programming knowledge in VLSI. My passion towards doing research in Machine Learning in extreme level. I want to take my research in a way where it helps people in all ways. He has done a few projects in Digital system design, Embedded system, CMOS VLSI technology which are real time based. One of the project is Arduino based heart rate monitoring system using heart beat sensor.



Challapalli Ujwala, Under graduate Student, Department of Electronics and Communication Engineering, Koneru Lakshmaiah Education Foundation (Deemed to Be University), Vaddeswaram, A.P., India. Area of Interests in VLSI, Machine Learning, Embedded in Designing (IoT) Internet of Things. Also, done some academic projects in the core competencies required in the VLSI domain and has the knowledge in the basic VLSI programming. Basic interests to do a research in Robotics and make sure that the technology helps people in all the possible ways. In this regard to know more about the design of basic Line following Robotic vehicle. Also done some of the academic projects using Embedded Systems that can be used in real time analysis such as weather monitoring system etc.