DETECTION OF DENDRITIC SPINES FROM HIGH-RESOLUTION MICROSCOPIC IMAGES

A thesis

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of

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By

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FACULTY OF ENGINEERING AND TECHNOLOGY
JADAVPUR UNIVERSITY

Certificate of Recommendation

This is to certify that the dissertation entitled "**Detection Of Dendritic Spines from High Resolution Microscopic Images**" has been carried out by Surajit Sasmal (University Registration No.: 160149 of 2021-2022,Examination Roll No.:MCA2340037) under my guidance and supervision and be accepted in partial fulfilment of the requirement for the Degree of Master of Computer Application. The research results presented in the thesis have not been included in any other paper submitted for the award of any degree in any other University or Institute.

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This is to certify that the thesis entitled "Detection Of Dendritic Spines from High-Resolution Microscopic Images" is a bonafide record of work carried out by Surajit Sasmal in partial fulfilment of the requirements for the award of the Degree of Master of Computer Applicationin the Department of Computer Science and Engineering, Jadavpur University during the period of December 2022 to May 2023. It is understood that by this approval, the undersigned does not necessarily endorse or approve any statement made, opinion expressed or conclusion drawn therein but approves the thesis only for the purpose it has been submitted.

……..

Signature of Examiner 1

Date:

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Signature of Examiner 2

Date:

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Declaration of Originality and Compliance of Academic Ethics

I hereby declare that this thesis entitled "Detection of Dendritic Spines from High-Resolution Microscopic Images" contains a literature survey and original research work by the undersigned candidate as part of my Degree of Master of Computer Application.

All information has been obtained and presented in accordance with academic rules and ethical conduct.

I also declare that, as required by these rules and conduct, I have fully cited and referenced all materials and results that are not original to this work.

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Acknowledgement

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Contents

Chapter 1

Introduction

Spine morphology and dissemination are imperative variables related with various neurological clutters such as mental hindrance and schizophrenia for spine morphological anomalies. In this manner, the visualization and examination of dendritic spines are fundamentally critical for investigate on synaptic versatility. Dendritic spines are the postsynaptic morphological specializations that get synaptic impulses.

It is broadly accepted that long-term synaptic versatility is went with by basic changes in neural connections, especially at dendritic spines. In 1995, Michele Father et al. [1] checked advancement variation within the dendritic spine within the essential culture of hippocampal neurons utilizing Confocal Laser Filtering Microscopy (CLSM). They watched the morphological changes of neurons over four weeks. In 1996, a study [2] appeared that the dendritic spine could be a energetic structure, profoundly responsive to changes within the surrounding condition. Dendritic spines alter shape and measure, and their turnover rates decay with age. The complex instrument behind memory creation and maintenance is accepted to join synaptic versatility within the hippocampus and cerebral cortex.

1.1 Dendritic Spines : Brain Activity and Its Relation to Neurological Disease :

Dendritic spines are the postsynaptic portion of most excitatory synapses in mammalian cells. Spinal mass accumulates rapidly during early postnatal development and decreases significantly as the animal grows into adulthood. Dendritic spines are central to information acquisition and storage; Understanding how spines are formed and preserved, especially in intact brains, could provide important insights into how the brain is able to learn and remember.

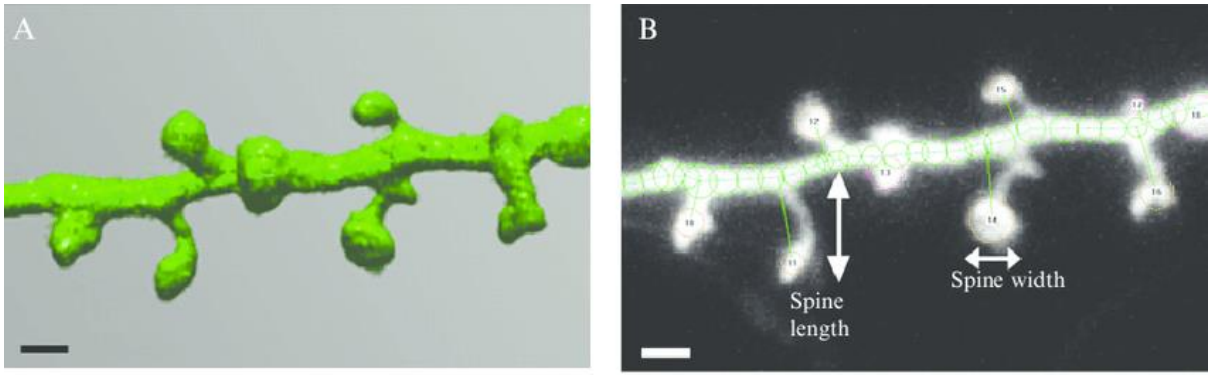

Figure 1.1 Sample image of Spine Morphology

(Image Courtesy:

Link: https://www.researchgate.net/figure/Spine-morphology-analysis)

Spinal cord morphology is associated with certain components of cognitive processes such as learning, eg. Data analysis and memory creation, i.e. long-term data storage. The interaction between neuronal activity and different signaling pathways can lead to new spinal cord growth and affect spinal cord growth and stabilization/pruning, a process important for learning and memory [3]. Impaired spinal dynamics can lead to mental and neurodevelopmental disorders. Alzheimer's disease (AD) is an age-related neurodegenerative disease whose disease is fatal in the elderly. The symptoms of AD are early memory loss, which is a progressive decline in cognition and cognitive function, followed by dementia [4]. Spinal abnormalities and abnormalities of the spinal cord, including shape change and reduced size, have been observed in patients with schizophrenia [5].
Mental illness has been associated with spinal cord injury as well as head weakness or head size.

1.2 Classification of Dendritic Spines :

Spine morphology and stability are strongly influenced by synaptic activity. In recent years, spinal morphology has shown a high degree of plasticity. In developing tissues such as organ cultures, transformation of the spine and reconstruction of postsynaptic density (PSD) occur within minutes [6]. Additionally, many studies have shown that these and other changes may result from or be due to synaptic activation. There are four types of spines: short, filopodia, cork, and protruding acanthus. During spinogenesis, when spines begin to form, they form

elongated shapes called filiform feet [7]. Filopodium is usually seen during growth. This species has a long neck and no head.

Over time, the filopodia are thinner, i.e. Long-necked and small-headed, blunt spines i.e. neckless or short ones and mature-like spines i.e. long-necked, large onion-headed spines [8]. Mushroom spines are considered large and generally stable spines and have larger spines containing neurotransmitter receptors and postsynaptic density (PSD), while filopodia are immature spines without synapses.

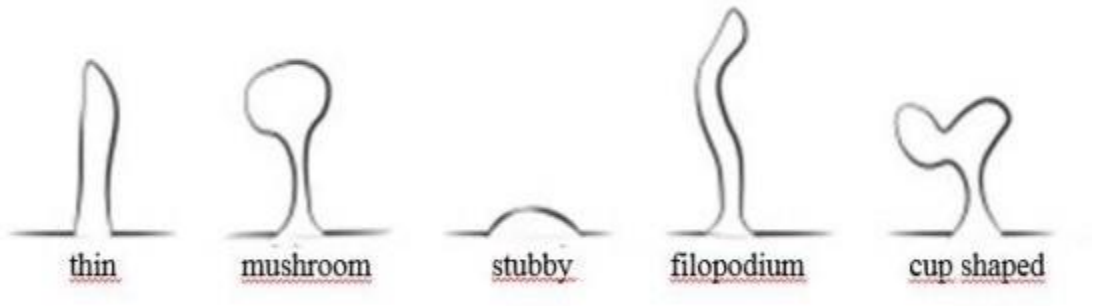

Figure 1.2 : Different Spine morphology

(Image Courtesy : site-ChargeTransfer

link :https://www.charge-transfer.pl/2018/05/preludium-for-studies-of-neurons/)

1.3 Imaging Modalities :

Advances in biomedical imaging technology have revolutionized biomedical research by enabling the visualization of multidimensional data. Biomedical imaging measures physical parameters such as density, concentration, texture, and area. There are many diagnostic tools available, including microscopy, ultrasound, computed tomography (CT), magnetic resonance imaging (MRI), and positron emission tomography (PET). Super-resolution microscopy provides optical access to the complex morphology of neurons in brain tissue and best resolves the details important for neuronal function. SpineJ is a semi-automated ImageJ plugin that facilitates fast, accurate and unbiased analysis of spine morphology, providing an intuitive and user-friendly graphical user-friendly interface [9].

However, dendritic spines have nanoscale structural details such as spines that are important but cannot be resolved by optical methods such as confocal and two-photon microscopy. Given

this limitation, the researchers turned to measuring fluorescence to estimate the size of the spinal cord. Advances in fluorescent microscopy are an important step towards improving the study of living cells. Use fluorescence microscopy to understand cell structure and function. The Confocal Microscope is used to examine the structure and morphology of cells and to distinguish abnormal cells for diagnosis.

In confocal microscopy, the optical or fluorescent properties of the sample are measured using a small pinhole to block all light, i.e. light seen only by the material in the confocal microscope. 3-DIMENSIONAL. plane [10].

Confocal laser scanning microscope (CLSM) uses laser light to scan a pixel and a piece of material.

spinal cord morphology was studied during normal development in mice using nanoscale stimulated emission extinction microscopy (STED) and tested the hypothesis that it is impaired in the mouse model of fragile X syndrome (FXS) [11]. Stimulated emission depletion (STED) microscopy overcomes the diffraction-limited resolution of confocal microscopy and effectively reduces the observation volume. Here, two synchronized laser pulses are used to eliminate the breakage problem [12].

1.4 Mouse and Human Models :

Humans and mice share many genes, and scientists can understand how humans work by studying the anatomy, physiology and metabolism of mice. Over the years, house mice have become the preferred animal model for genetic research. At the sequence level, about half of human genomic DNA can be sequenced as mouse genomic DNA [13].

In the early days of biomedical research, scientists created mouse models by selecting and breeding specific mice to produce offspring with specific needs. Now, scientists are using mice as genetic models of human diseases to study their development and test new treatments.

As a research tool, mice helped advance research and led to the development of important new drugs. Like other animals, rats are born with life-shortening diseases such as high blood pressure, cancer, diabetes, and osteoporosis. But some diseases that affect humans, such as cystic fibrosis and Alzheimer's disease, don't usually affect mice. To test this disease in mouse

models, the disease was induced in mice by manipulating the mouse genome and environment, e.g. Mice are genetically engineered for this purpose [14]

1.5 Motivation

Extensive research has been done in the field of dendritic spine analysis. However, there is sti ll room for improvement. All of these methods work to some degree, but in any case more bo oks need to be done. Current methods often involve manual labor and require researchers to s pend a lot of time and effort analyzing the spine. That's why we need an automated method th at minimizes user interaction and delivers the desired results.

1.6 Scope Of The Current Work :

The major focus of this thesis report is to detect the dendritic spines in two-dimensional (2D) dendritic spine images of different time stamps of rat dissociated hippocampal cultures.The scope of the work in dendritic spine detection encompasses the development and implementation of techniques and algorithms to identify and analyze dendritic spines, which are small protrusions on the dendrites of neurons. This field involves various aspects such as image processing, computer vision, and machine learning. The primary goal is to accurately detect and quantify dendritic spines in microscopic images or neuronal reconstructions. This work contributes to understanding the morphology and function of neurons, investigating neurological disorders, and advancing neuroscience research.

1.7 Organisation Of Thesis :

- In Chapter 1 discusses a basic introduction to dendritic spines and their importance in our memory formation and in different diseases. Similarities between the mouse model and the human model are also discussed.
- In Chapter 2 is a brief survey of different work done to analyze the dendritic spine's shape and nature and how we get our motivation for work.
- In *Chapter 3* all the basic theoretical concepts related to the automatic Spine analysis, i.e. 3D to 2D transformation
- *In Chapter 4* discuss different types of detection algorithms and analyze experimental results.
- In chapter 5 Conclusion of the thesis.

Chapter 2

Literature Survey

This research focuses solely on experimental studies examining the properties and structure of dendritic spines. Research on the learning mechanisms of the brain has been going on for more than a century. In 1888, Cajal first described dendritic spines discovered using a silver impregnation protocol developed by Golgi. In the earliest form of this argument, Cajal predicted that learning requires the growth of new neurons (Ramon y Cajal 1893).

Tanzi (1893), meanwhile, reiterated Spencer's (1862) earlier suggestion that changes in existing connections would cause information to be stored in the brain.

In 1973 it was found that brief tetanic stimulation produces a long-term synaptic plasticity, long-term potentiation (LTP) in the mammalian hippocampus that can last for hours or days (Bliss & Lømo 1973) [15].

Later in 1982 Hopfield effectively linked LTP to the neural network theory of brain function because it uses local rules, an important feature of neuronal coordination and enables multiple objects, making neural networks very useful for calculating aesthetic properties [16] .

2.1 Literature survey on the classification of spines:

A synapse is a zone of specialized contact between two neurons, serving to transmit information from cell to cell. Most synapses are formed between the axonal bouton and the dendritic spine, which is a specialized protrusion from the dendritic membrane. Dendritic spines come in a variety of shapes and sizes, differing greatly across brain areas, cell types, and animal species (Ghani et al., 2017). During structural analysis dendritic spines are traditionally grouped into four fixed classes according to their morphological features reflecting head and neck properties: mushroom, thin, stubby, and filopodia (Figure 2.1). Mushroom spines have a large head and a small neck, separating them from a dendrite. They form strong synaptic connections, have the longest lifetime, and therefore are thought to be sites of long-term memory storage (Hayashi and Majewska, 2005; Bourne and Harris, 2007). Thin spines have a structure similar to the mushroom spines, but their head is smaller relative to the neck.

They are more dynamic than mushroom spines and believed to be “learning spines,” responsible for forming new memories during the synaptic plasticity process, accompanied by head enlargement (Hayashi and Majewska, 2005; Bourne and Harris, 2007).

Stubby spines typically do not have a neck. They are known to be the predominant type in the early stages of postnatal development but are also found in small amounts in adulthood, where they are likely formed due to the disappearance of mushroom spines (Hering and Sheng, 2001).

Filopodia are long, thin dendritic membrane protrusions without a clear head, commonly observed in developing neurons. These spines may also be found in mature neurons, but under specific conditions, for example, induction of plasticity after different types of brain injury (Yoshihara et al., 2009). Compared to other types of dendritic spines, filopodia are very mobile and flexible structures with a short lifetime.

On electron micrographs, filopodia in most cases do not have PSD and the neighbouring axonal terminal contain only a few synaptic vesicles, indicating that they are not likely to form functional synapses. Because of this, filopodia are usually excluded from spine counts during synaptic density calculation (Berry and Nedivi, 2017). There are also additional spine shape classes which have been named by different research groups such as branched and cup-shaped spines (Maiti et al., 2015)[17]

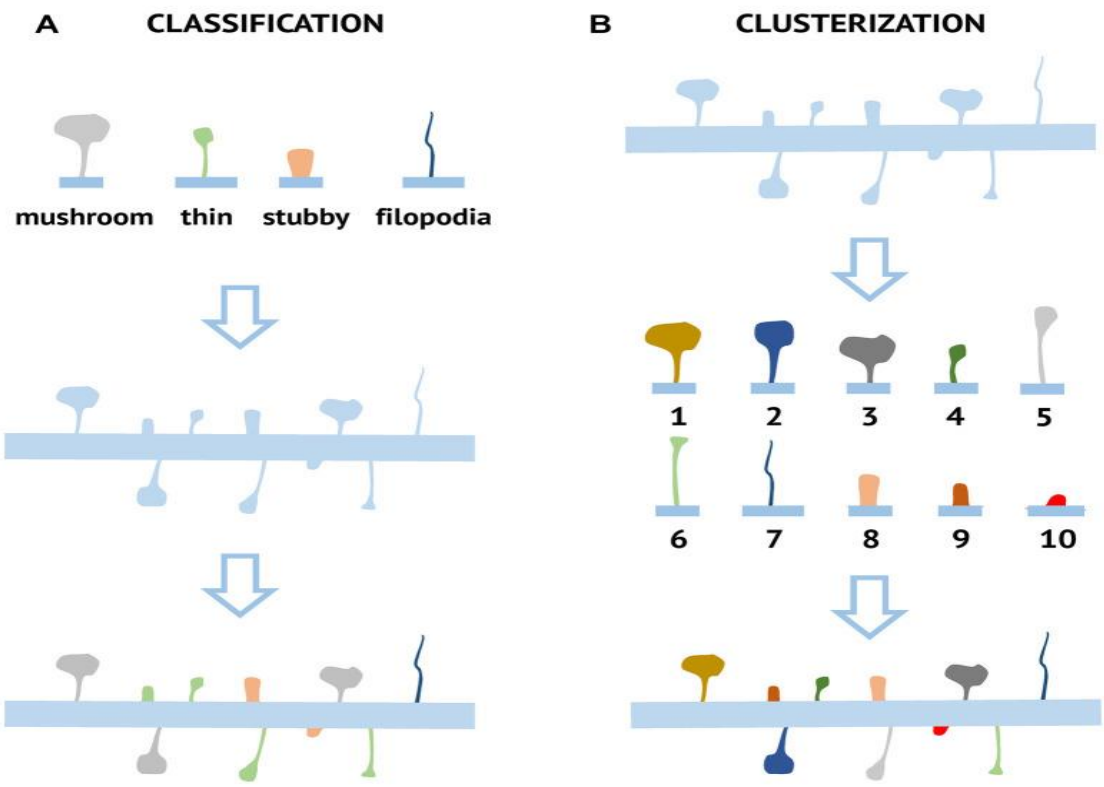

Figure 2.1 :Classification & Clusterization of different types of Spines

(Image Courtesy: site : NCBI

link : https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7561369/figure/F1/)

Comparison of classification and clusterization pipelines used for the analysis of dendritic spine morphology. For classification approach **(A)** several possible morphological spine types (mushroom, stubby, thin, and filopodia) are defined based on pre-determined criteria. Each spine is then assigned to one of these classes based on numerical morphological criteria. For the clusterization approach **(B)**, spines are grouped into clusters based on their common morphological features. Ten different clusters (1–10) are shown as an example, but the number of different clusters and parameters used for clusterization depends on the particular algorithm and dataset.

2.2 A Literature review on dendritic Spine Connectivity and its Impact on Mental Health :

The link between mental illness and altered dendritic spines was first discovered in 1974 (Purpura, 1974). Purpura suggests that mental disorders are associated with abnormally long thin and short bones of dendrites of cortical neurons in unconscious children [20].

A. Alvarez et al. [21] reviewed some of the spine research and reconstruction and concluded that the shape and size of dendritic spines change and their flexibility decreases with age.

Hosokawa et al. [22] performed an analysis of changes in the length of individual spines using confocal microscopy. They used confocal microscopy of hippocampal slices, in which a custom design "DiI-droplet technique stains single CA1 pyramidal cells." Synaptic enhancement is caused by "drug LTP" produced by the use of perfusion solutions containing Ca2+, reduced Mg2+ and tetraethylammonium.

Using this16 experimental approaches , the authors observed (small) elongation in the patient's bones and reported several changes in the nasal tissue in the spine. In 1992, Harris et al. shows that the number of spines nearly doubles from the 15th postnatal day to adulthood. However, this doubling is not observed uniformly in all spines: slender spines, mushroom spines have perforated PSD and spinal apparatus, and branched spines increase approximately fourfold, while the number of other species decreases or does not change [twenty-three].

Also, in 1999 Kirov and Harris showed that adult hippocampal neurons can form new bone. Filopodia is rarely seen in the old hippocampus.

However, inhibition of synaptic transmission in hippocampal slices causes growth of filopodia and nascent spines, possibly compensating for the loss of synaptic activity [24].

In 2014, Rafael Yuste [25] demonstrated the behavior of dendritic spines from the activity of the circuit and found that spikes equip the circuits with the activity of neural networks.

Roberto Araya [26] used two-photon calcium imaging of rat neocortical pyramidal neurons to examine the relationship between spinal morphology opened by small synaptic stimulation and the excitatory postsynaptic potential it produces. They show that spike time simulations induce synaptic potentials and selectively shorten neck length.

2.3 Literature Survey on automated tools invented for spine analysis:

Research articles on electronic devices designed for spinal cord monitoring:

W. Christopher Risher et al. [27] developed a RECONSTRUCT software that successfully reconstructs the spinal cord during development of the primary visual cortex in mice.

They use the Golgi-cox staining protocol, take images and export their z-scores to an image analysis program. Although it can be a powerful tool for studying neuronal infrastructure, Golgi staining has several disadvantages.

Perhaps the biggest barrier to widespread acceptance of this approach is the time commitment involved. Another disadvantage of Golgi-Cox staining is the variability of the spinal cord among analysts. Individuals may have different spine classifications even when describing the same dendrites. This work makes it difficult, if not impossible, to compare the results of different analysts, which can be a major problem for experiments that require large amounts of data described by many.

2016 Basu et al.[28] proposed a semi-automated method called 2D-Span for the quantitative analysis of spinal morphological changes with less intervention. This tool is useful in many applications involving large-scale descriptions of dendritic spines and allows rapid and accurate analysis of changes in the spinal cord. They used confocal microscopy images of dendritic spines from isolated hippocampal cultures. For spine analysis, users should select the dendrite segment by drawing two points, use the generated convolution core to cut the spine segment, and finally draw the eager spine to remove features such as spine length and head width with high precision. and minimal interference. The method counts the number of dendritic spikes and the distribution of four types of segmented dendritic spines (stumpy, cork, filopodia, and spine head protrusions).

This approach allowed us to document major changes in dendritic spine area and length, two of the most important aspects of spinal cord plasticity. 2dSpan requires less user involvement than the work of Ruszczycki et al. [29]. Therefore, many spines can be identified quickly and easily without affecting the accuracy of the predicted spine. The operation depends on the user's selection of the dendritic region of interest.

A general binaryization is then performed on the image, sometimes leading to poor results and incorrect segmentation of the spine of interest.

Assoc. TW et al. [30] developed the Imaris software for four-dimensional (4D) image analysis and automated neuronal tracking. With Imaris for Neuroscientists, users can manage and organize successful experiments, including graphs and data analysis.

The strength of Imaris lies in its high performance data IMS, which guarantees good performance even with very large 3D files (terabyte range). The accuracy of automatic tracks was manually checked and only tracks longer than 60 seconds were included in the analysis. While Imaris is well suited for examining the entire spine, it cannot model the 3D morphology of individual spines due to manual intervention.

Peng Shi et al. [31] proposed a new semi-supervised learning (SSL) method to determine the online morphological distribution of dendritic spines.

In their method, dendritic spines are detected and segmented from dendrites according to the wavelet transform. The spinal cord symptoms are then removed and SSL is applied. However, the entire effectiveness of this method depends on the features and parts the neurobiologist chooses for training.

Janoos et al. presented an algorithm for 3D reconstruction and identification of dendritic spines. [32].

For the quantitative analysis of spine plasticity, they performed a 3-dimensional segmentation of spines using the multi-aperture (MSO) [35] method to determine 3-dimensional morphological features of individual spines. They used confocal microscopy images of dendritic spines from isolated hippocampal cultures and brain slices. Although his methods do not involve the spine, as it takes more time, it is still effective.

2.4 Recent research on dendritic spine morphology and its relationship to age and brain:

In 2019, Benjamin D. Boros and M. Greathouse conducted a study in which they observed and digitally reconstructed the three-dimensional morphology of dendritic spines in the dorsolateral prefrontal cortex in intelligent humans aged 40-94 years. Their study used a randomized controlled trial to establish the association between spinal cord injury, age, Mini-Mental State Examination (MMSE) scores, and Alzheimer's disease (AD) pathology. They found a link between the spine and aging in humans, similar to findings in other animals.

In addition, the presence of AD pathology was associated with increased spine length, decreased spine diameter, and increased filopodia density. Their research shows how spinal morphology in the prefrontal cortex changes with aging in humans and highlights the key features of these changes in the spine. individuals with. to work. They concluded that there is still room to improve the performance of the tool, especially with regard to the user interface and further steps [36].

N. Ofer and D.R. Berger analyzes the regeneration of rat neocortical neurons using computer algorithms. They found that most of the spine can be separated into head and neck, providing a precise measurement of the neck of the spine. They investigate the presence of different diseases by using information about spinal morphological parameters. Their analysis revealed a correlation between postsynaptic density and spinal cord volume.
In addition, they found no evidence for different morphological subtypes of the spine, suggesting that spine length and head volume are regulated independently [37].

In 2020, L. K. Parajuli and M. Koike review research that allowed us to understand the three-dimensional fine structure of synapses by focusing on dendritic-spine synapses in the rat brain. They discuss several important studies demonstrating differentiation of spines, principles in dendrites, patterns of presynaptic connectivity, and activity-based modulation [38].

C. Ortiz-Sanz and A. Gaminde-Blasco conducted a study in 2020 to investigate the events that lead to synaptic changes. They applied oligomeric Aβ to ex vivo models of primary hippocampal neurons and rat organotypic hippocampal cultures to observe spinal cord changes using high-resolution images and algorithm-based analysis.
Their findings showed that the stability of the three types of spines was significantly reduced after treatment with Aβ oligomers. Interestingly, they observed an increase in the density of all dendritic spines in Aβ-treated organotypic hippocampal slices, this increase driven mostly by the increase in short spines, while the mushroom-shaped and thin spines were absent [39].

In 2021, J. Choi and Sang-Eun Lee presented an integrated visualization tool for 3D dendritic spine mapping called DXplorer. The system supports 3D rendering of the spine and plots high-end features extracted from the 3D mesh of the spine.

Users can interact with each other, search and identify them based on advanced features. The authors report high-dimensional morphological features derived from dendritic spine 3D networks. They also developed an interactive machine learning system with visual search and user feedback, using an interactive 3D grid view to further classify spinal cord phenotypes [40].

2022, S.J.Kim and Y. Woo discovered a novel function of Rai14 (retinoic acid-inducible protein 14), an F-actin-binding protein in dendritic spine dynamics. Their research showed that Rai14, located in the neck of the spine, controls the spine.

Chapter 3

Image Pre-processing Modalities :

Image preprocessing is the step of formatting an image before it can be used inferential. This preprocessing includes resizing, orientation and color change of the image. Preprocessing requires cleaning the input image data for each model. Identifying the right preprocessing steps is the most important step to improve performance, which requires a deep understanding of the problem and data collection. Here, converting 3D images to 2D images is one of the important steps in image preprocessing.

3.1 3D to 2D Conversion :

It is possible to reconstruct a 2D focus image that adequately represents all the information in the 3D group. The 3D group contains all the information needed to reconstruct the 2D image. To create a 2D image from a 3D group, projection in the z direction is generally preferred, as it contains the most information and does not require interactive processing. More than 80% of the scientific community uses MIP to reduce a 3D mass to a 2D image. MIP stores the maximum level for all x and y positions along the z axis.

The image of the 3D group is called the measurement map, and the image of the reference value corresponding to this measurement map is called the projection. Perform Gaussian noise removal on 2D MIP images to improve quality. MIP is recommended as it is the simplest z-projection method without parameters, fast and easy to use, like the NIH Fiji / ImageJ software

tool. Our data were generated using light-induced imaging of different images of neurons in hippocampal cultures isolated from mice before and after chemical long-term exposure (cLTP).

❖ Image Pre-processing steps are given below :

➢ Steps to convert a 3D image to stack splitting:

i. Plugins →LSM toolbox →Open LSM (See Figure 3.1 and 3.2)

ii. Image →Stack →Tools→ Stack splitter (See Figure 3.3 and 3.4)

iii. Sliced Image Save as→ Tif (See Figure 3.5)

iv. Opening the saved Image with IrfanView Software (See Figure 3.6)

v. After converting the image to a grayscale image and adjusting the colour (see figure 3.7)

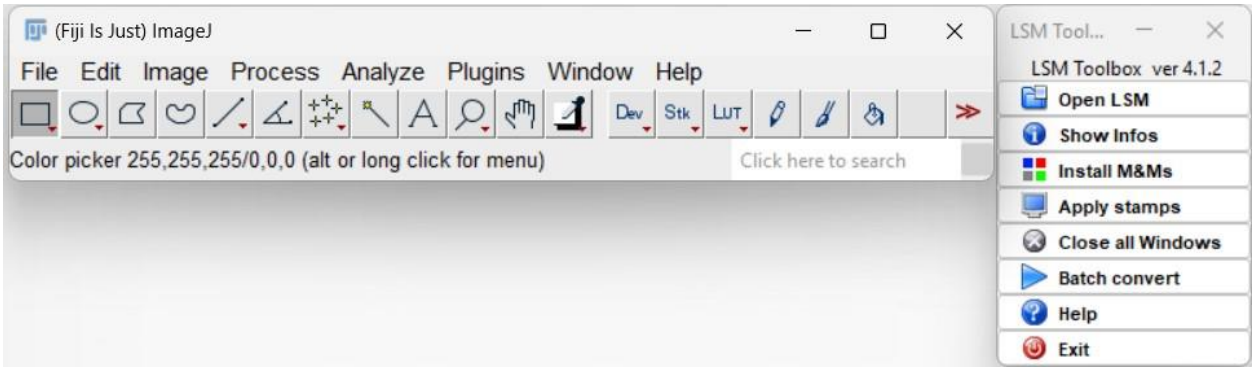

Fig 3.1 Open the LSM toolbox in Fiji Software(Refer to step i above)

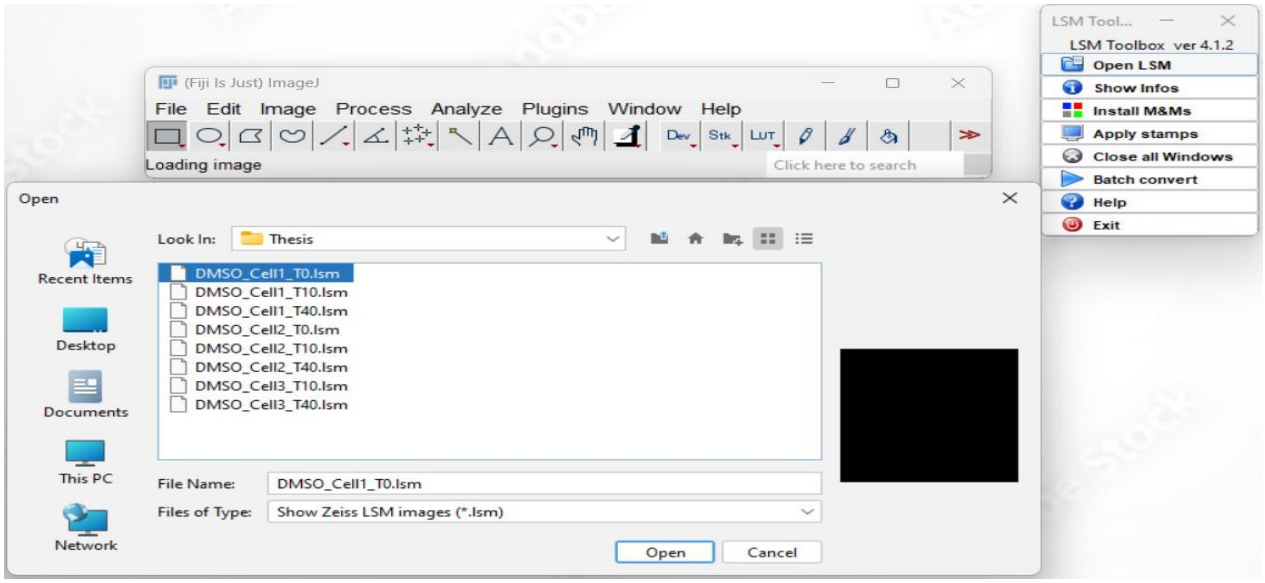

Fig 3.2 Open LSM image (Refer to step i above)

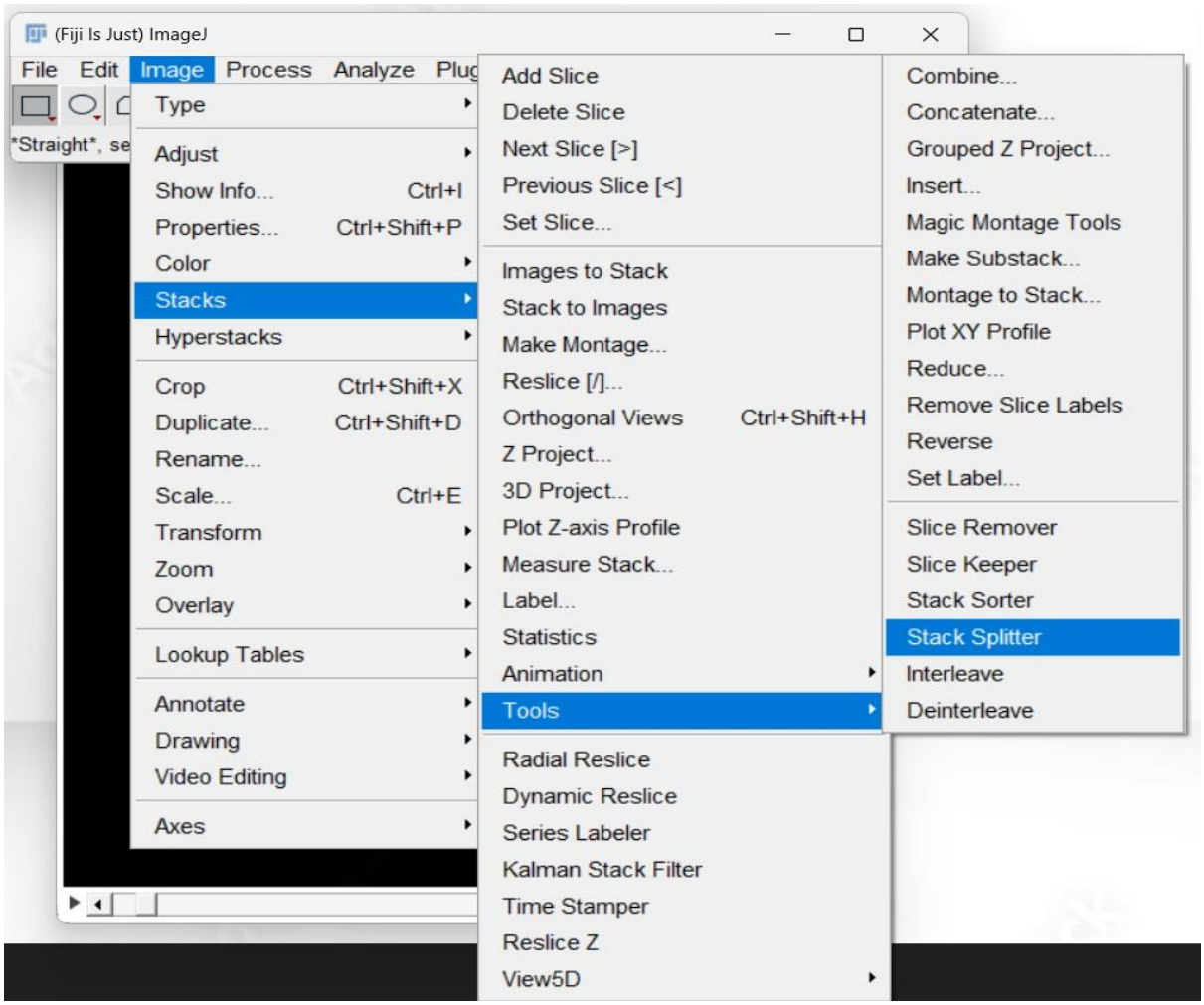

Fig 3.3 Navigate to stack splitter (Refer to step ii above)

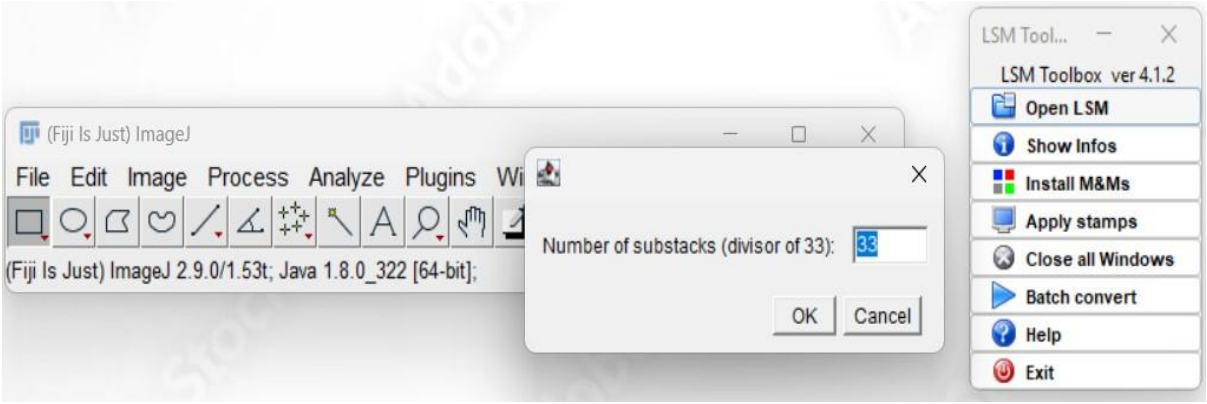

Fig 3.4 Stack Splitting of a 3D image (Refer to step ii above)

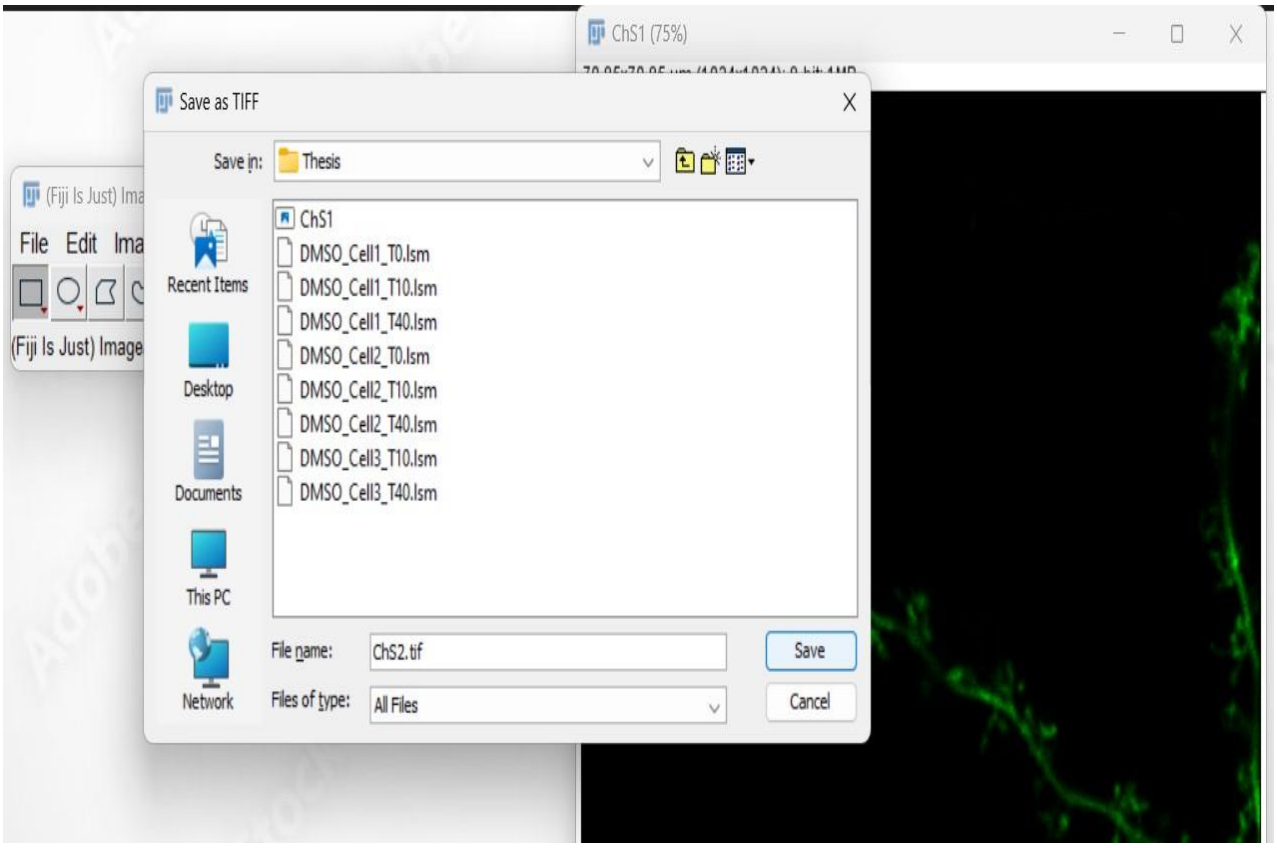

Fig 3.5 Saved the Sliced image (Refer to step iii above)

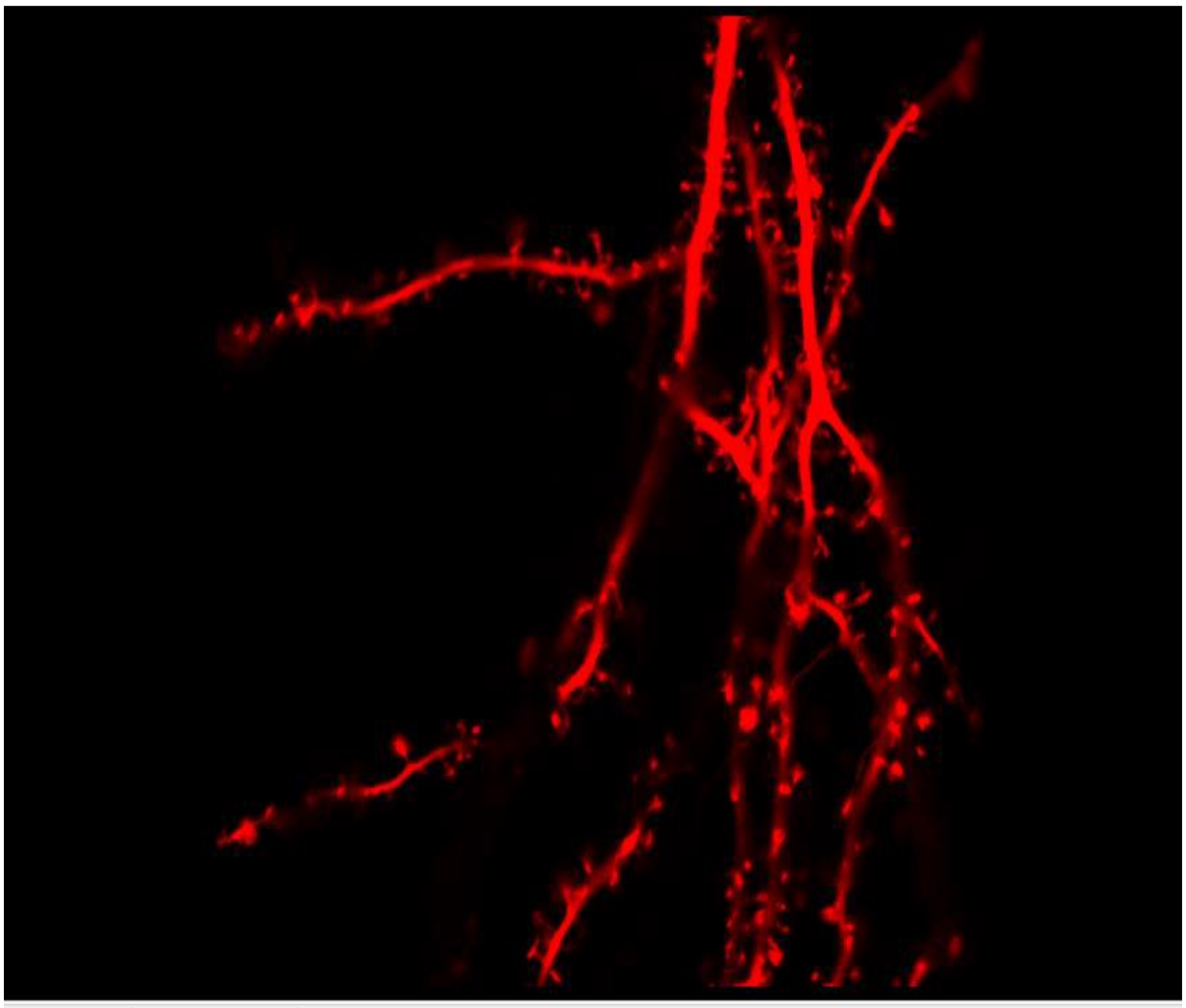

Fig 3.6 Open Saved image in Irfan view Software (Refer to step iv above)

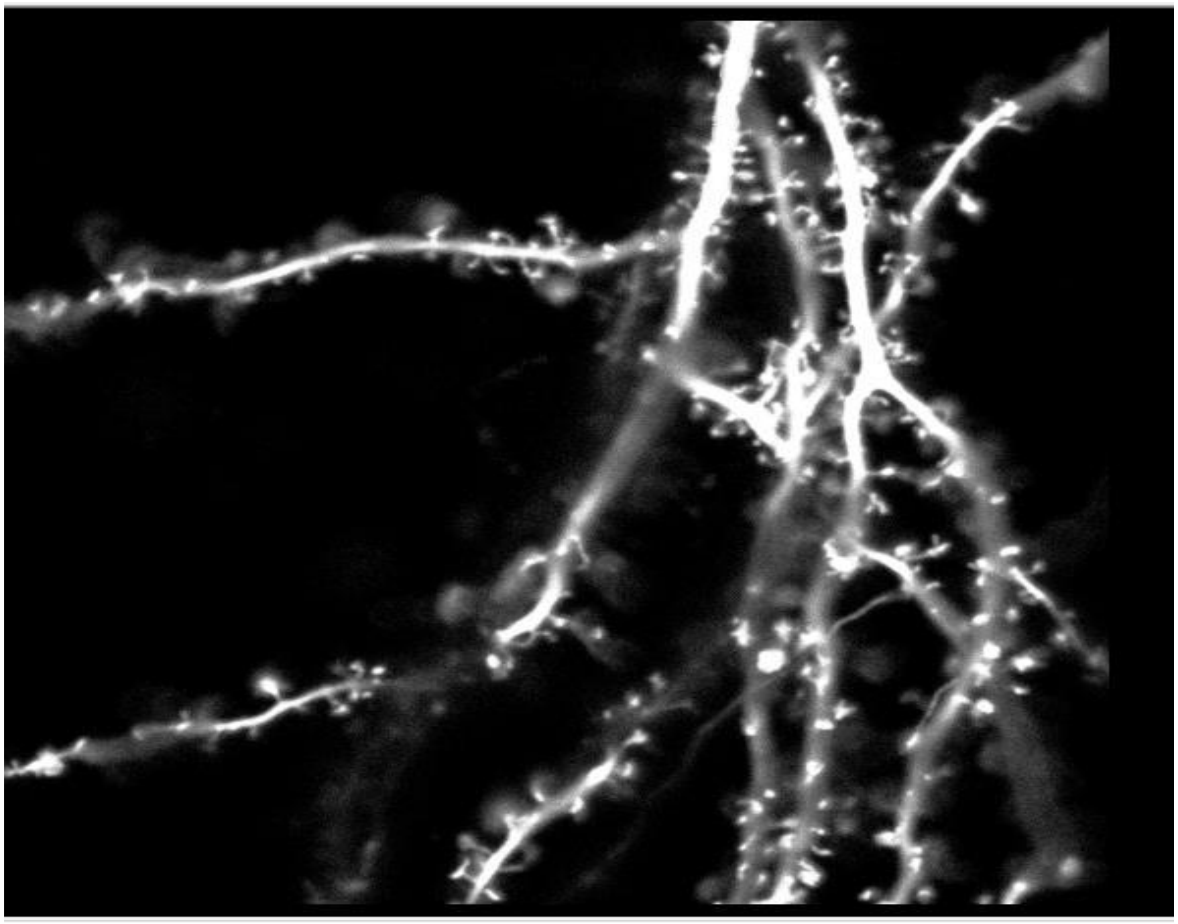

Fig 3.7 Converting the image to a grayscale image Refer to step v above)

- Steps for 3D to 2D image using MIP concept :

  i) Process→Filter→3D Gaussian Blur (See Figure 3.8)

  ii) Tiff Image→Type→8 bit (See Figure 3.9)

  iii) Image→Stacks→Z Project (See Figure 3.10)

  iv) Save Z-projection image (See Figure 3.11)

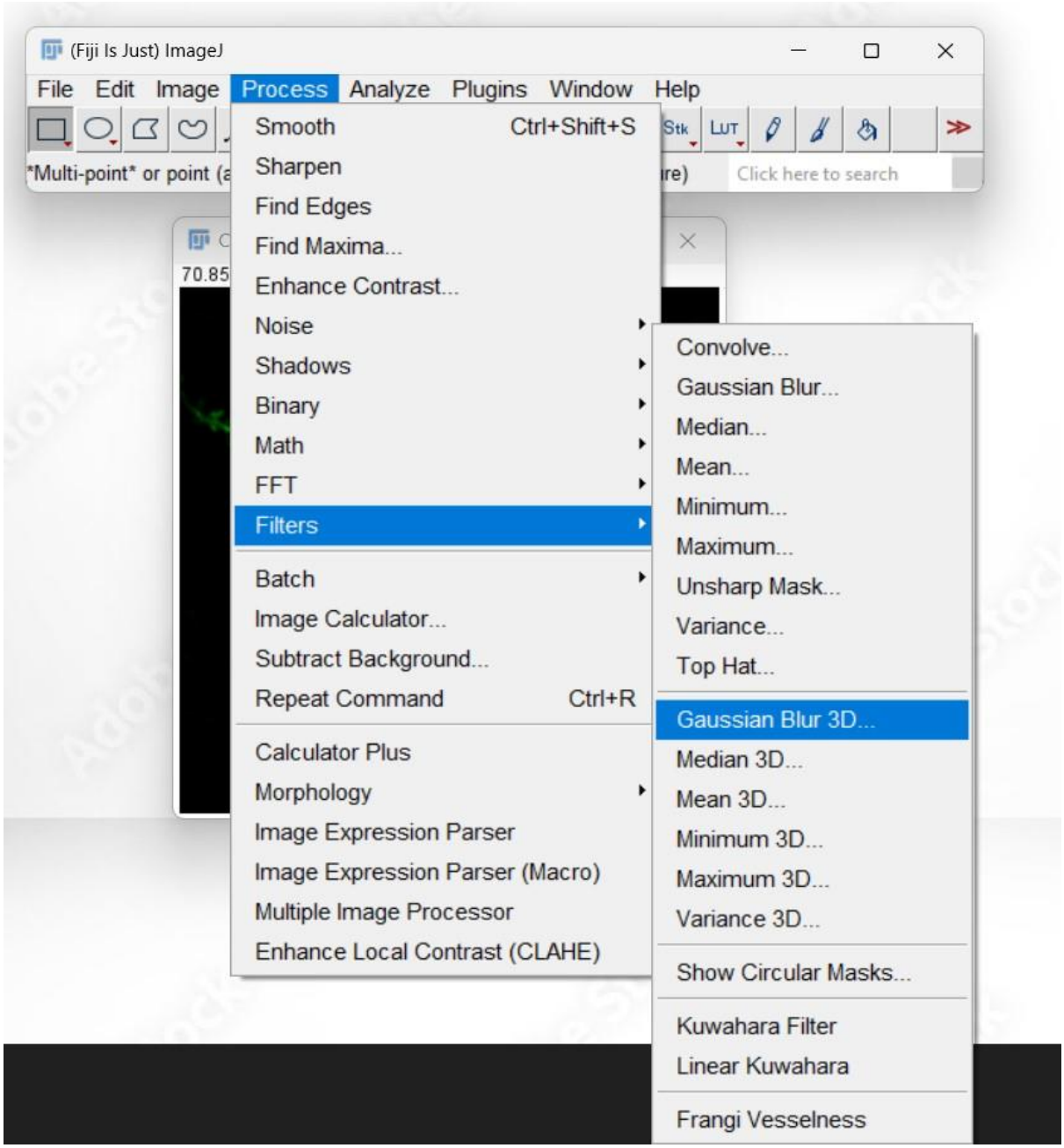

Fig 3.8 Applying Gaussian blur 3D filters (Refer to step i above)

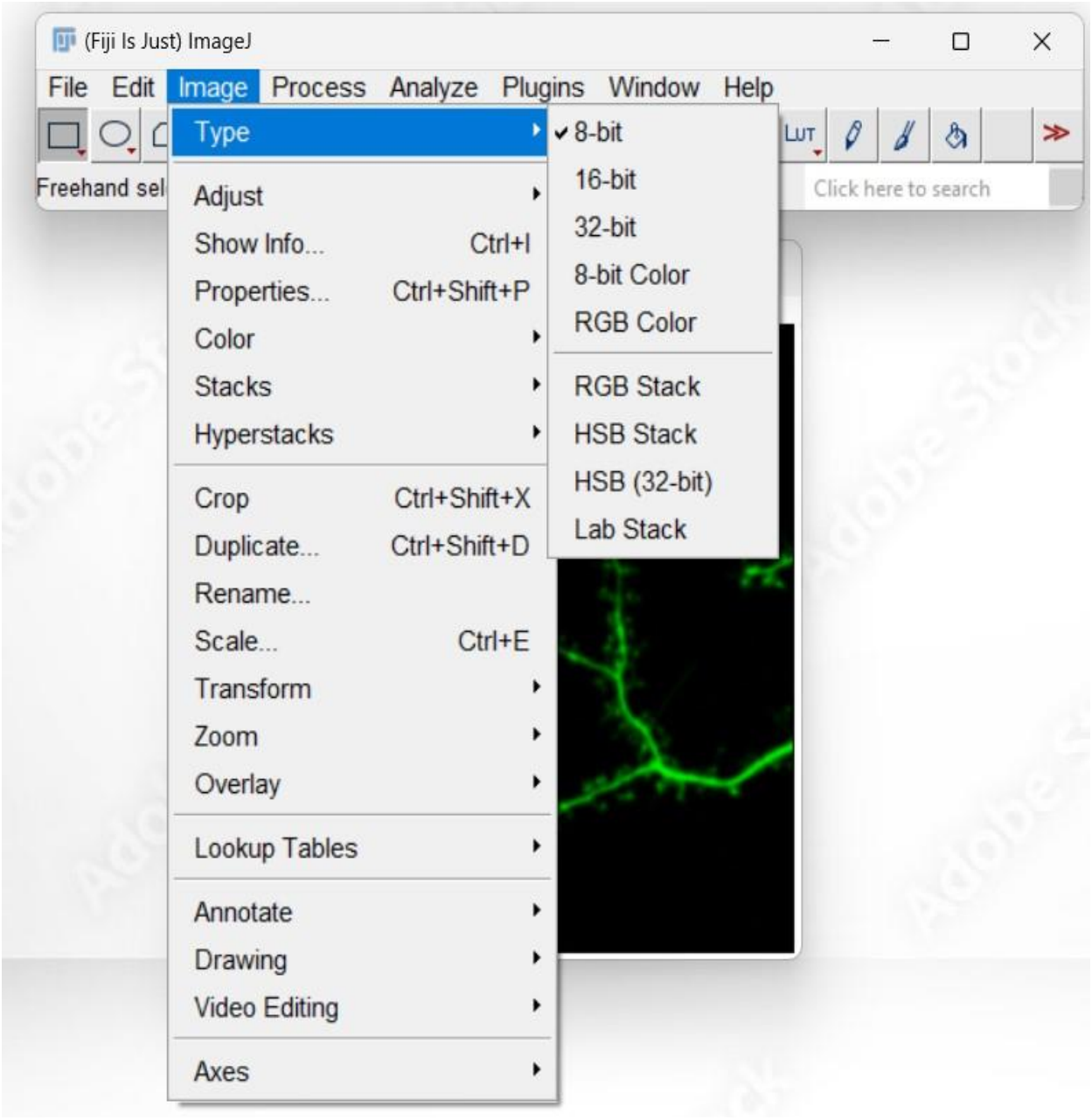

Fig 3.9 TIF image (Refer to step ii above)

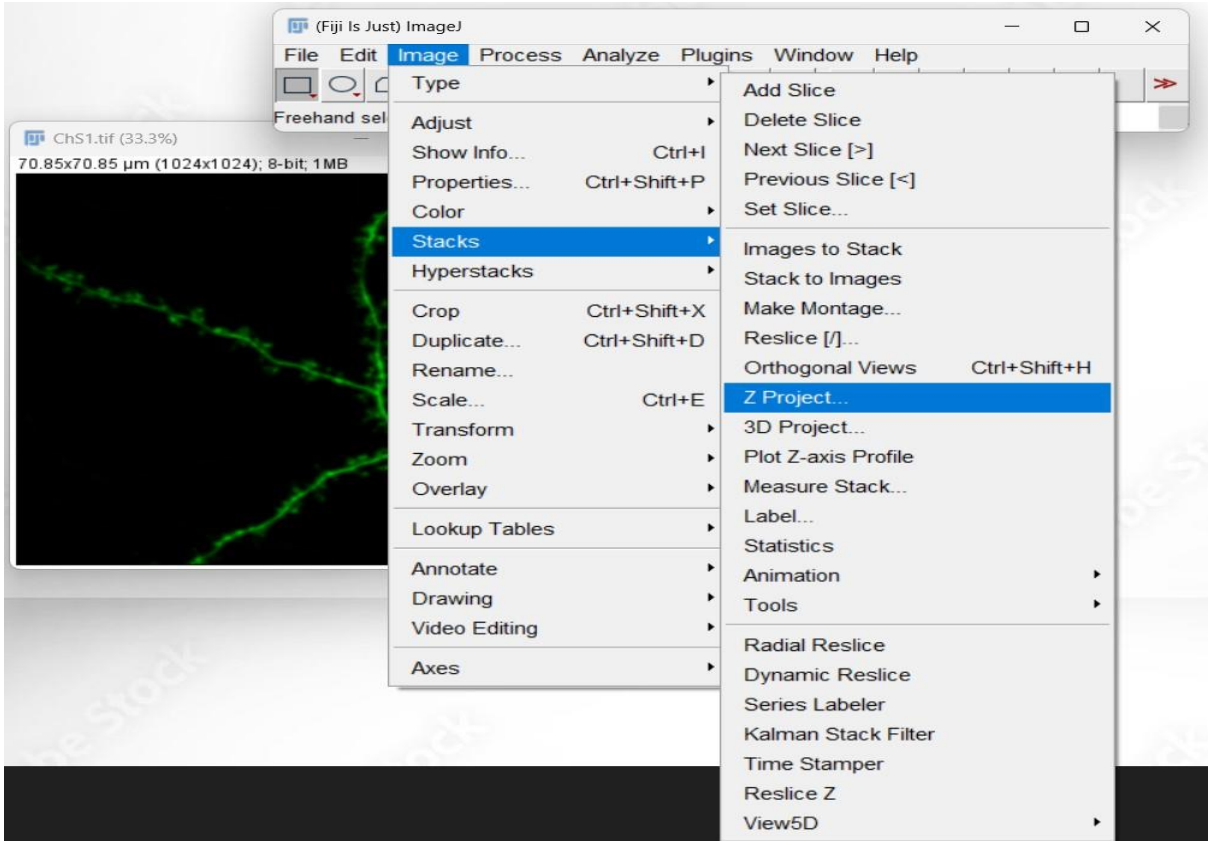

Fig 3.10 Applying Z-project (Refer to step iii above)

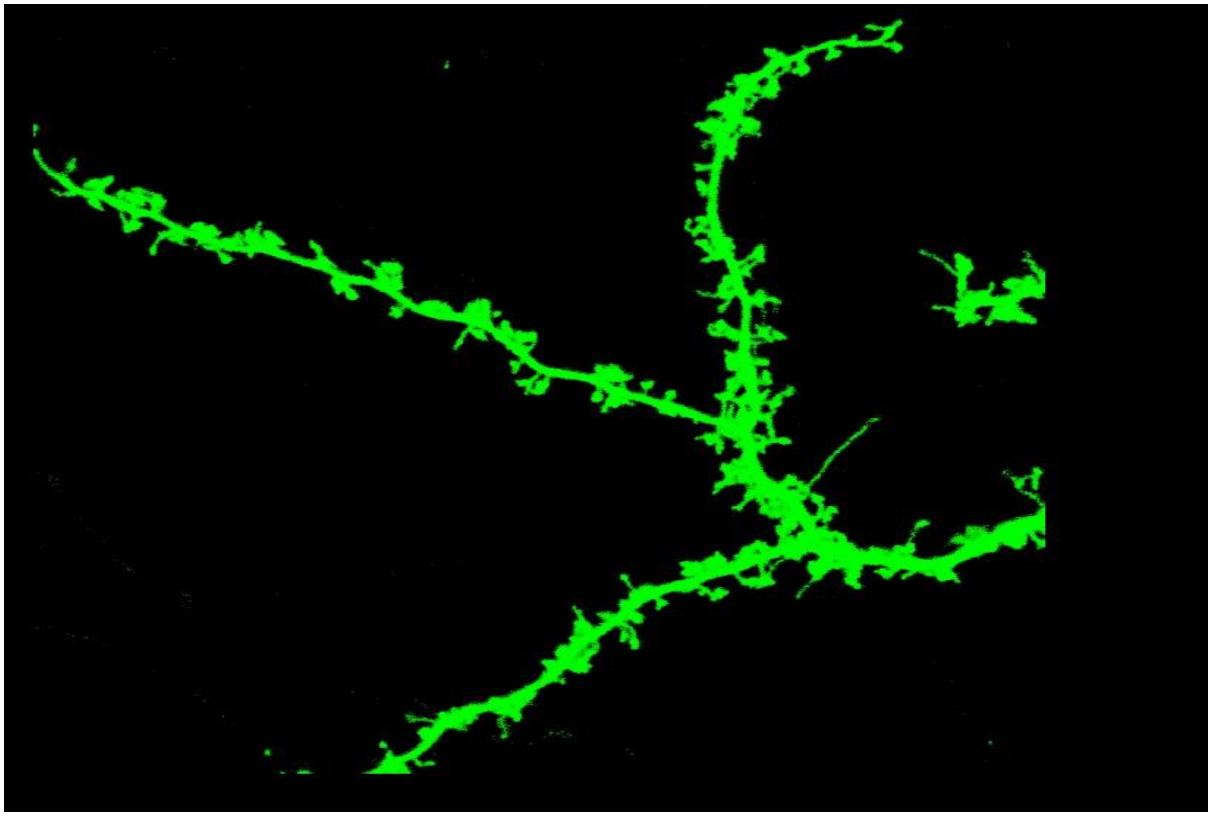

Fig 3.11 Image after MIP using Z-projection (Refer to step iv above)

Chapter 4

Dendritic Spine Detection:

Dendritic spines are small protrusions along the branches of neurons that play a crucial role in synaptic connectivity and information processing in the brain. Accurate detection and analysis of dendritic spines from high-resolution microscopic images are essential for understanding their structural and functional properties. This study presents an automated approach for dendritic spine detection from high-resolution microscopic images.

The proposed method leverages state-of-the-art deep learning techniques and image processing algorithms to achieve robust and precise detection of dendritic spines. Firstly, a deep convolutional neural network (CNN) is trained using a large annotated dataset to learn discriminative features of dendritic spines. The trained CNN is then utilized to classify image patches and identify potential spine candidates.

The automated detection of dendritic spines from high-resolution microscopic images enables accelerated research in neuroscience, facilitating large-scale analysis and quantitative investigations. The proposed approach contributes to the understanding of brain function, synaptic connectivity, and the underlying mechanisms of learning and memory. Furthermore, it provides a foundation for future studies and opens up opportunities for advancements in neurological research and drug discovery.

4.1 Hand Annotation and Dataset:

To create custom datasets for detecting dendritic spines, we utilize MakeSense.ai, an open-source and free tool designed for object detection annotation. MakeSense.ai provides an intuitive interface and a range of annotation tools, simplifying the process of annotating images for object detection tasks.

By leveraging MakeSense.ai, we can upload our microscopy images containing dendritic spines and define the annotation tasks specific to our requirements. The platform supports various annotation types such as bounding boxes or polygons, allowing us to accurately outline and label the dendritic spines in our images.

The following steps are involved in the process of creating custom datasets for dendritic spine detection using MakeSense.ai:

i. Get started and upload images: Begin by signing in to MakeSense.ai and uploading the microscopy images containing dendritic spines. (See fig 4.1)

ii. Object detection and edit labels (spines): Select Object detection option and edit the labels name (See fig 4.2)

iii. Draw rectangles and select name (Spines): draw rectangles around the detected spines and assign the label name "Spines" to them. (See fig 4.3)

Export Annotation in YOLO format: Export the annotations in the YOLO format, which is compatible with the YOLO object detection framework.

By following these steps, you can effectively annotate and export your custom datasets in the YOLO format for dendritic spine detection using MakeSense.ai.

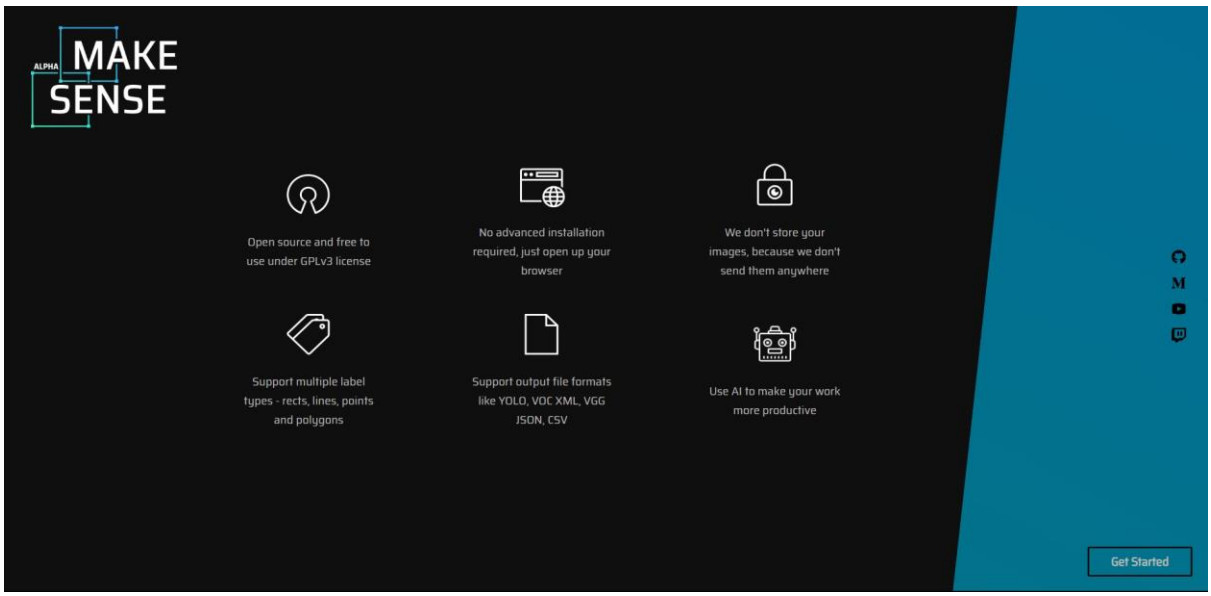

Fig 4.1 makesense.ai and Get Started to make annotation

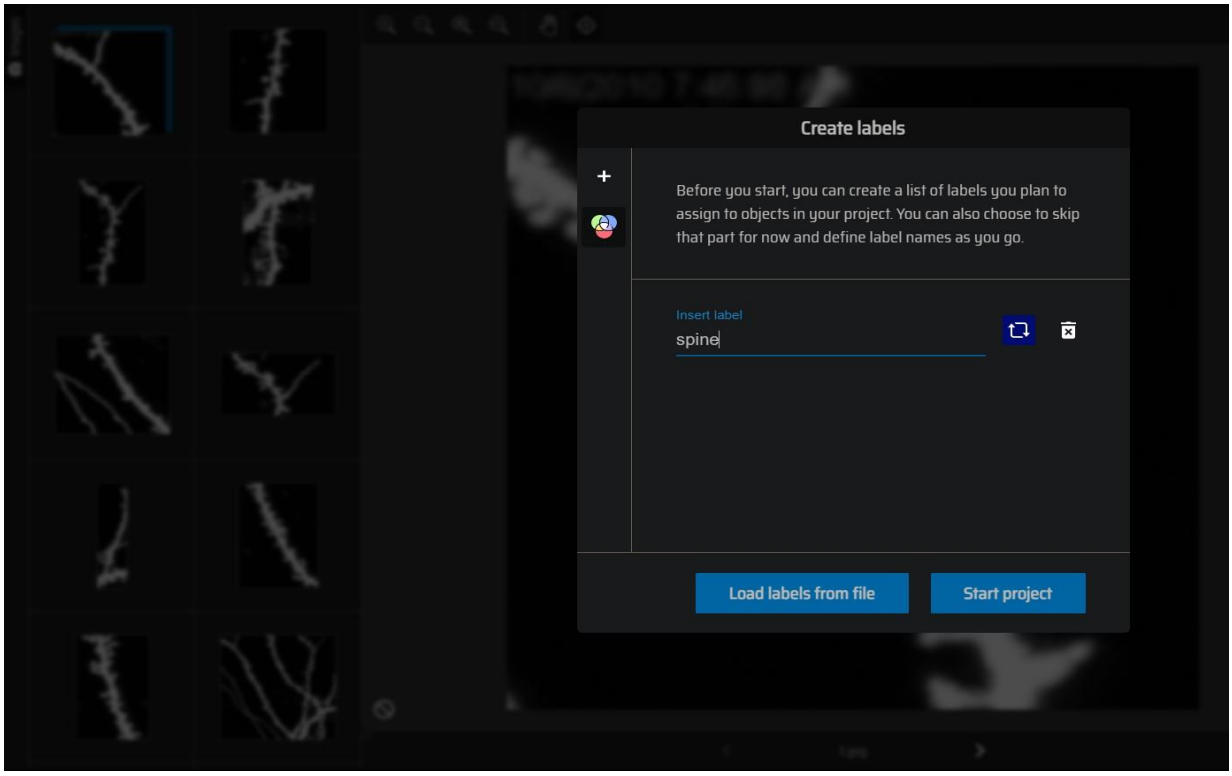

Fig 4.2 Create labels name and start project

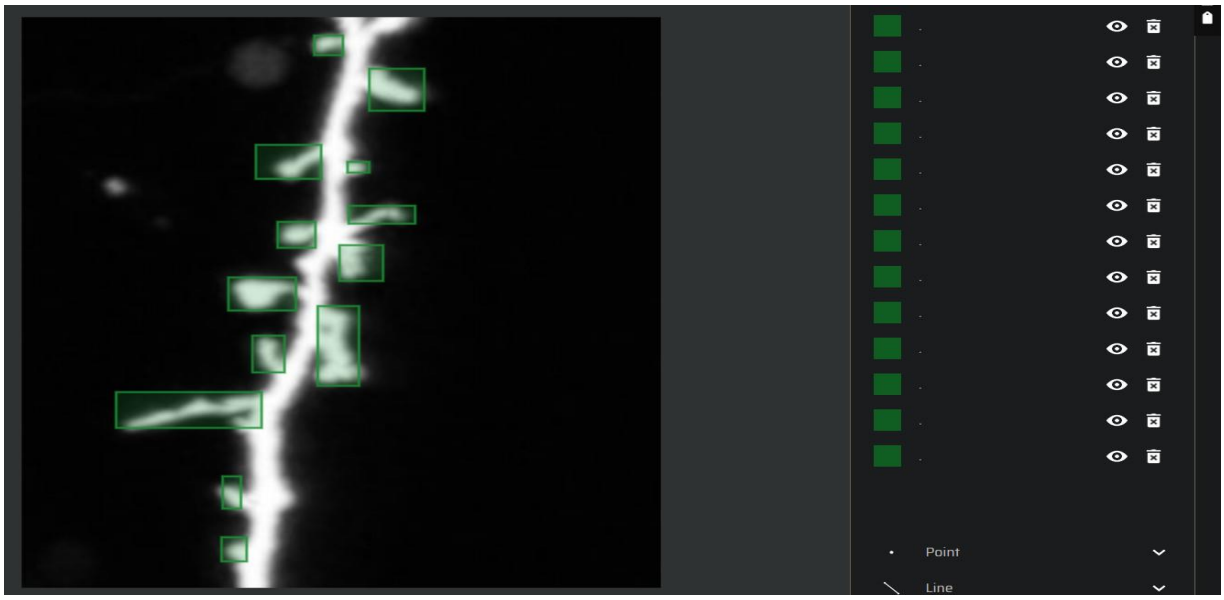

Fig 4.3 Draw rectangles

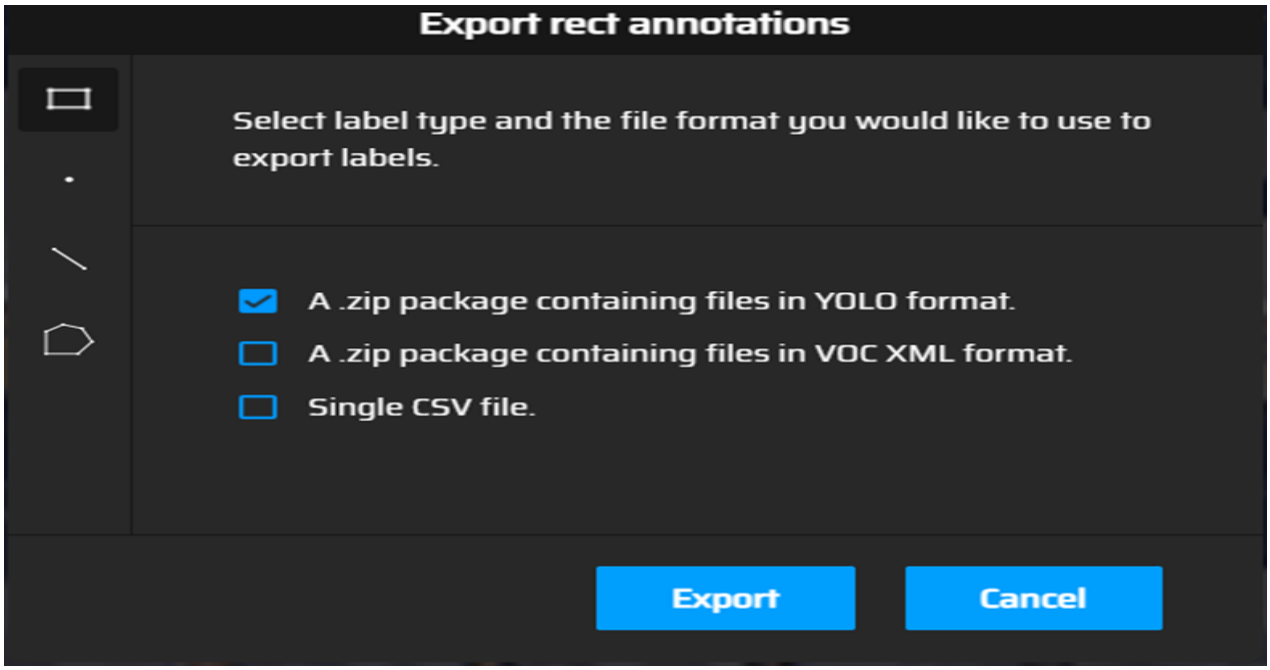

Fig 4.4 Export Annotation in YOLO format

(Source : Site link - makesense.ai)

4.2 Detection Algorithm:

Object detection algorithms are computer vision techniques that aim to identify and localize objects within images or videos. They enable various applications, including autonomous driving, surveillance, and object recognition.

Different types of object detection algorithms include region-based approaches like Faster R-CNN, which utilize region proposal networks (RPNs) to generate potential object bounding boxes. Single-shot approaches like YOLO (You Only Look Once) achieve real-time detection by dividing the image into a grid and predicting bounding boxes and class probabilities directly. Other algorithms, such as SSD (Single Shot Multi Box Detector) and Retina Net, strike a

balance between speed and accuracy. Each algorithm has unique characteristics, and the choice depends on the specific requirements of the task at hand.

4.3 "You Only Look Once": YOLO Detection:

The "You Only Look Once" model [18] considers bounding boxes and can achieve a mean average precision (mAP) of 63.4% on the PASCAL VOC 2007 dataset. The model frames object detection as a regression problem to spatially separated bounding boxes and associated class probabilities.

A single network predicts bounding boxes and class probabilities directly from full images in one evaluation. Since YOLO's entire detection pipeline operates as a single network, it can be optimized end-to-end directly on detection performance. The framework was attractive for our problem because it uses a global approach (rather than sliding window and regional analysis) to encode contextual information about classes when making predictions.

The YOLO detection network has 24 convolutional layers followed by 2 fully connected layers. Alternating 1 x 1 convolutional layers reduce the features space from preceding layers

It divides the image into an even grid and simultaneously predicts bounding boxes, confidence in those boxes, and class probabilities. At test time, the conditional class probabilities and the individual box confidence predictions are multiplied together as follows:

$$Pr(Spine|Object) \times Pr(Object) \times IOUgt = Pr(Dendrite) \times IOUgt$$

Notably, YOLO, like work done in [19], has real time frame rate at test time.

- YOLOv5 is an advanced object detection algorithm known for its real-time performance and accuracy. It divides the input image into a grid and predicts bounding boxes and class probabilities. YOLOv5 comes in different variations: YOLOv5s (small), YOLOv5m (medium), YOLOv5l (large), and YOLOv5x (extra large). These variants offer a trade-off between speed and accuracy, with larger models providing higher precision at the cost of increased computational complexity. Users can choose the YOLOv5 variant that best suits their specific

requirements, considering the balance between real-time detection and optimal performance.

(Source : YOLO V5

GitHub Page link : https://github.com/ultralytics/yolov5#pretrained-checkpoints)

4.4 An Overview Of the YOLO v5 Architecture:

Object detection, a use case for which YOLOv5 is designed, involves creating features from input images. These features are then fed through a prediction system to draw boxes around objects and predict their classes.

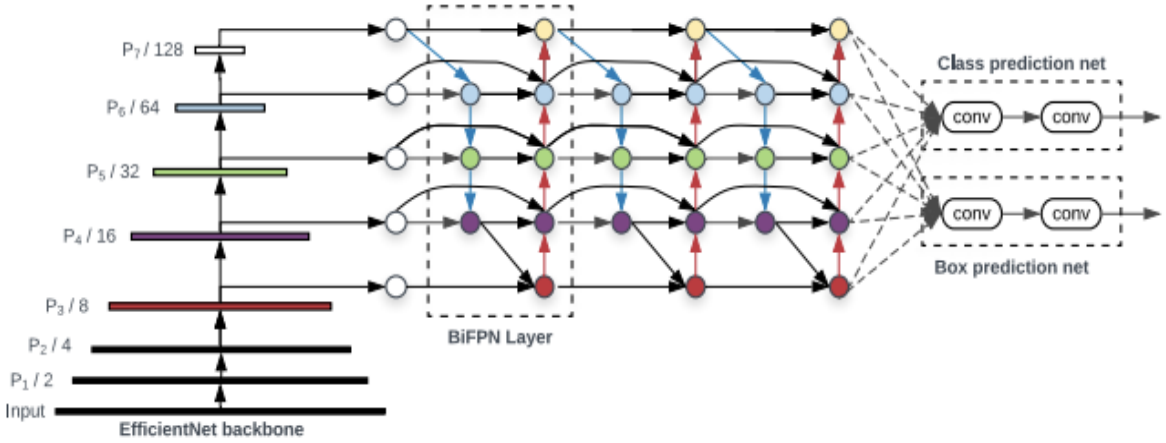

Fig 4.5 The anatomy of an object detector

The YOLO model was the first object detector to connect the procedure of predicting bounding boxes with class labels in an end to end differentiable network.

The YOLO network consists of three main pieces.

- Backbone: A convolutional neural network that aggregates and forms image features at different granularities.
- Neck: A series of layers to mix and combine image features to pass them forward to prediction.
- Head: Consumes features from the neck and takes box and class prediction steps.

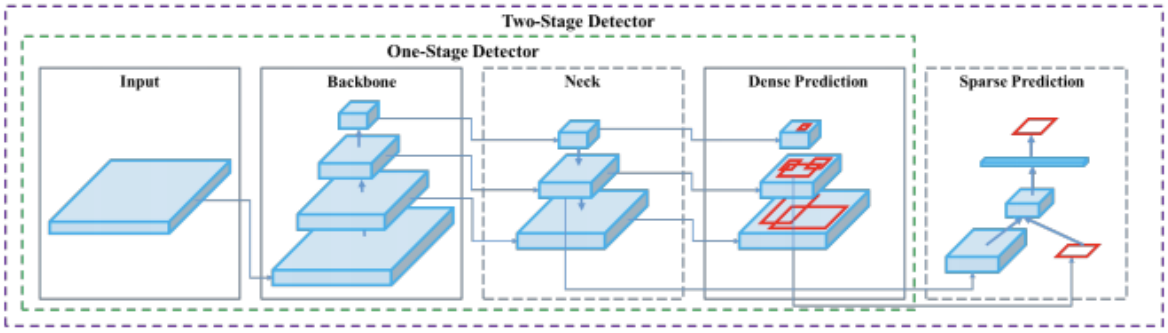

Fig 4.6 Another picture of the object detection process

The YOLO v5 architecture improves upon its predecessors by introducing architectural enhancements, such as the neck and multi-scale prediction, to achieve better accuracy and efficiency in real-time object detection tasks.

4.5 Application of YOLO and Experimental Results:

YOLO (You Only Look Once) [18] is primarily known for its applications in general object detection, it can also be utilized for dendritic spine detection in microscopy images. Dendritic spines are small protrusions along neuron branches, and detecting and analyzing them is crucial for understanding neuronal morphology and synaptic connectivity. By training a YOLO model specifically on annotated microscopy images of dendritic spines, it can effectively detect and localize these structures in new images. This enables researchers to study dendritic spine properties, quantify changes in spine density, and analyze the impact of neurological disorders or treatments on synaptic connectivity. YOLO's real-time capabilities and accuracy make it a valuable tool for dendritic spine detection and neuroscience research.

By training the YOLOv5 model on a dataset of annotated microscopy images containing dendritic spines, it can learn to detect and localize these structures in new images. This can facilitate automated analysis of dendritic spine properties, such as density, morphology, and dynamics, aiding in neuroscience research and understanding synaptic connectivity. Customizing YOLOv5 for dendritic spine detection requires careful dataset preparation, appropriate labelling, and fine-tuning of the model to ensure accurate and reliable results.

➢ Sample of Experimental Result:

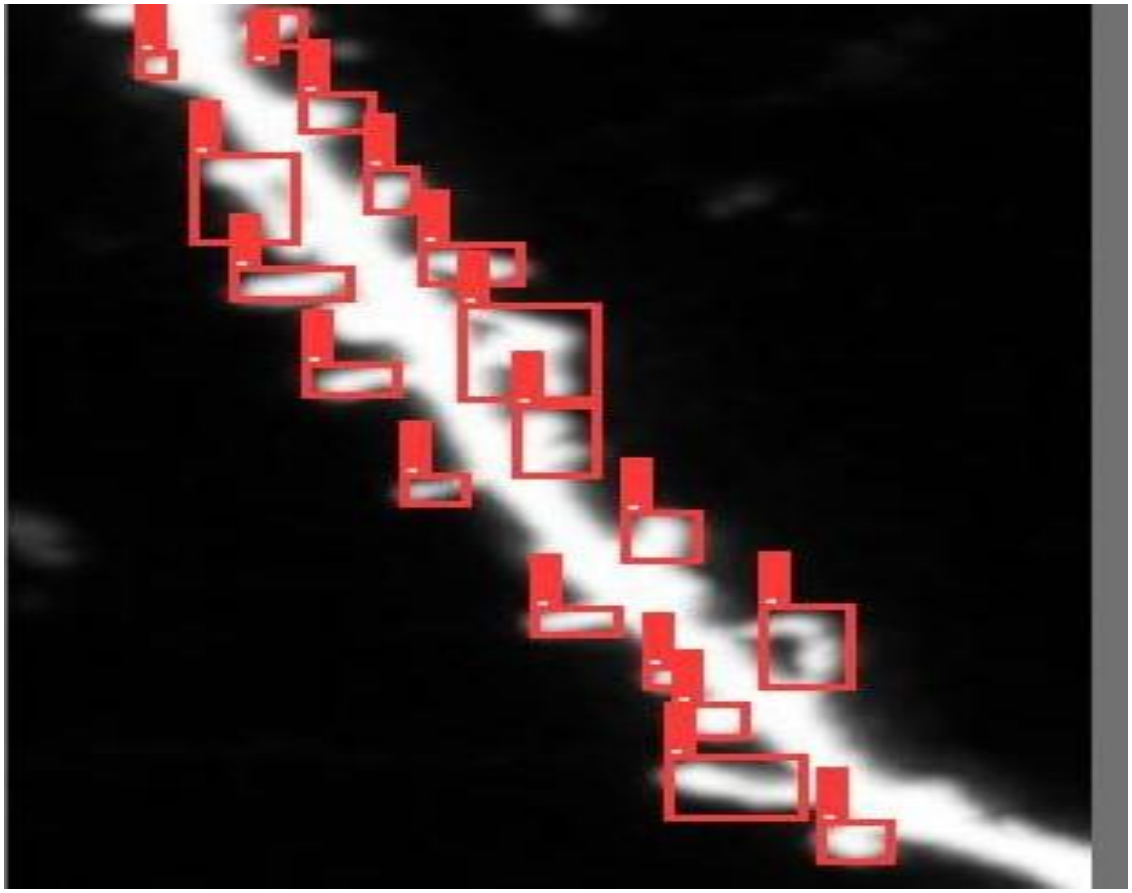

Fig 4.7 Dendritic Spine detection result

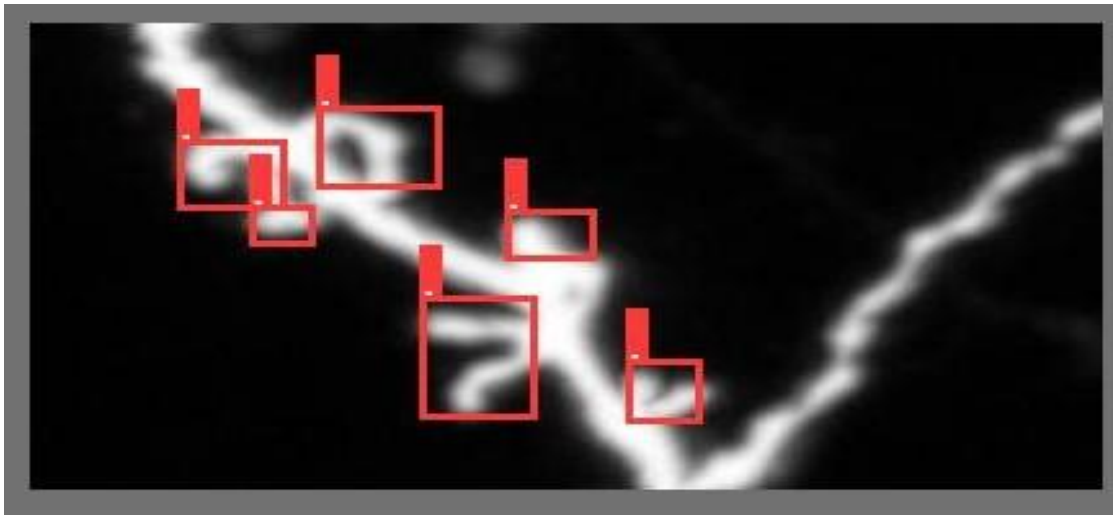

Fig 4.8 Dendritic Spine detection result

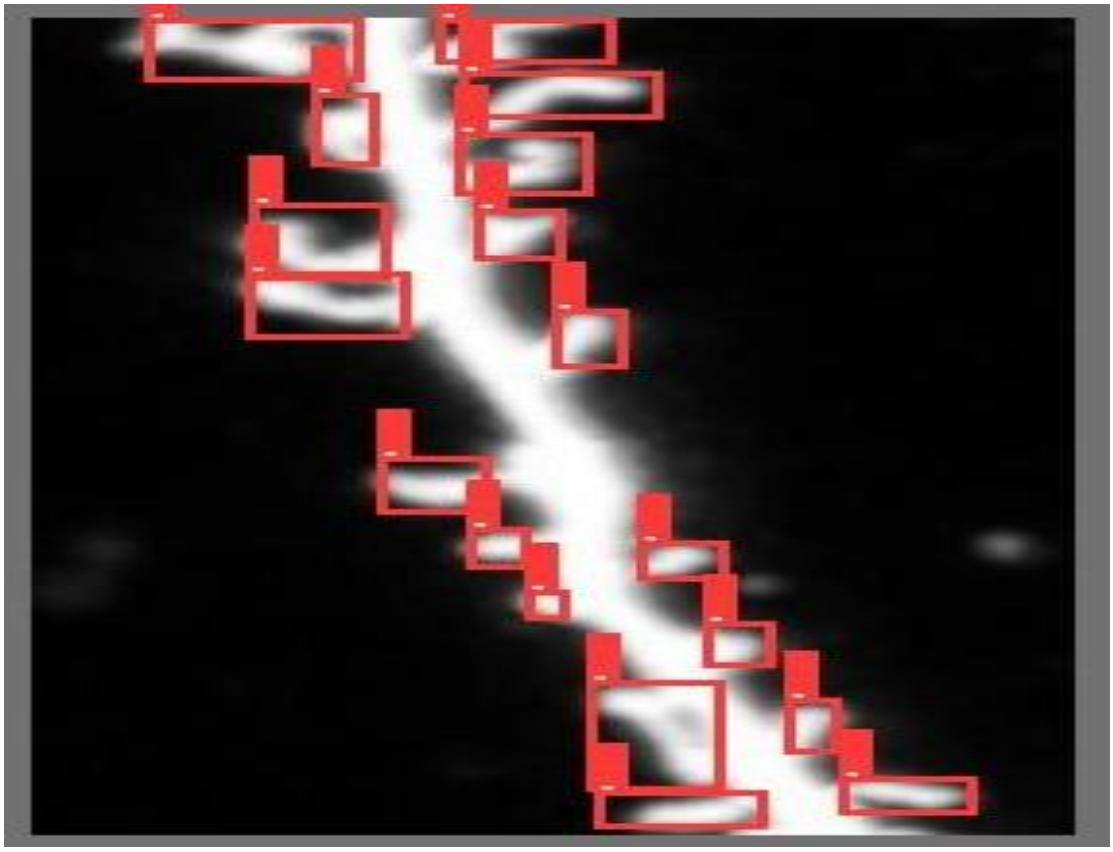

Fig 4.9 Dendritic Spine detection result

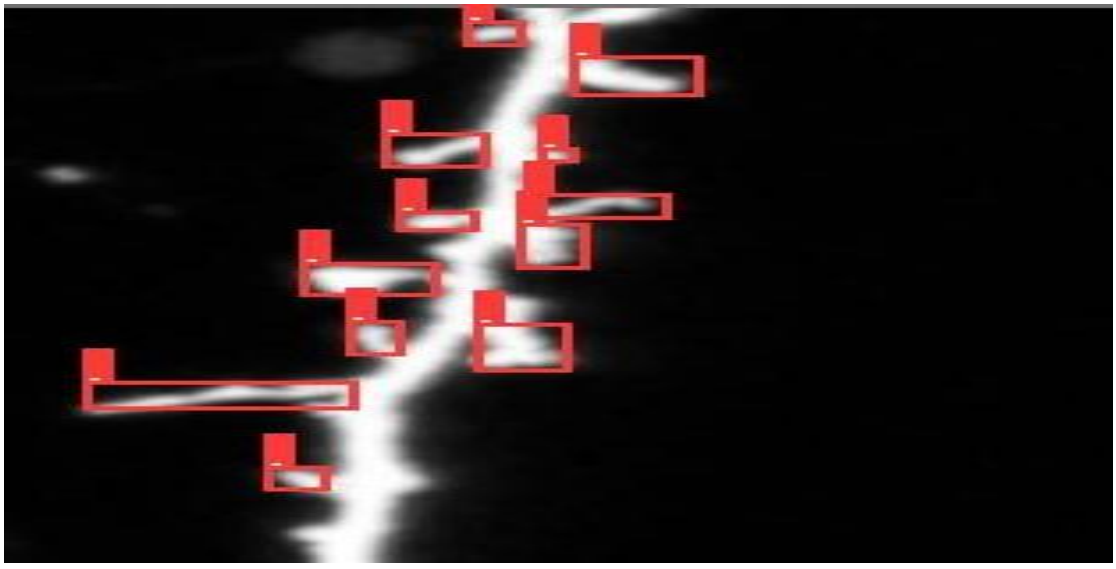

Fig 4.10 Dendritic Spine detection result

Chapter 5

Conclusion

This thesis introduces the theory and algorithm of dendritic spines detection from high resolution microscopic images. We review several methods of dendritic spine detection, reconstruction and analysis, along with a brief discussion on some of the digital topology applications that can be used for better performance of spine analysis methods. This study has explored the application of the YOLOv5 algorithm for the detection of dendritic spines in high-resolution microscopic images. The use of YOLOv5 has showcased its effectiveness in accurately identifying and localizing dendritic spines, which are crucial structures in neuroscience research.

By leveraging the strengths of YOLOv5, such as its real-time processing capability and the ability to handle objects at various scales, we have achieved promising results in dendritic spine detection. The algorithm's multi-scale prediction and anchor box mechanism have proven valuable in effectively capturing spines of different sizes and shapes.

Different experiments have shown that under stress or pathological conditions, spinal muscles show abnormalities such as shrunken or shrunken spine, the spine is immature, its size is reduced, the difference between vertebrae, ectopic formation and sometimes the number of vertebrae increases. This method can be used to identify changes in spine and spine, number and length, in different stress patterns or diseases such as Alzheimer's disease, schizophrenia and fragile X syndrome (FXS). It can be used in different neurobiological studies.

However, it is important to note that there are still areas for improvement in dendritic spine detection using YOLOv5. While the algorithm performs well in many scenarios, further refinement and optimization can be pursued to enhance its accuracy and robustness. Additionally, exploring the integration of additional techniques, such as

post-processing methods or incorporating contextual information, may yield even better results.

In conclusion, the utilization of YOLOv5 for dendritic spine detection demonstrates its potential as a valuable tool in neuroscience research. Through further advancements and refinement, YOLOv5-based approaches hold promise for advancing our understanding of dendritic spine morphology and their functional implications in neurological processes.

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