CLASSIFICATION OF SPINES: A CNN-BASED APPROACH FOR ROI DETECTION AND CATEGORIZATION

A PROJECT REPORT

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BONAFIDE CERTIFICATE

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Abstract

This work proposes an innovative approach for the classification of dendritic spines, essential components in neural signal transmission. Leveraging a two-pronged approach that combines a YOLO-NAS model for object detection and an InceptionV3-based convolutional neural network (CNN) for classification, the method categorizes spines into four distinct types: mushroom, thin, stubby, and filopodia. The primary objective is to establish a robust tool capable of not only categorizing dendritic spines but also detecting their structural components within larger neuron images, furthering investigations into the role of dendritic spines in diverse neurological disorders. Emphasizing the importance of advanced technology, this work significantly contributes to ongoing efforts aimed at enhancing our understanding of neural mechanisms.

Keywords

Dendritic spines · Morphological analysis · Neural signal transmission · Synaptic connectivity · Spine segmentation · Classification of spines · Neurological disorders

Contents

List of Figures

1 Introduction

Dendritic spines are minute protrusions that receive excitatory synaptic input and compartmentalise postsynaptic responses. Heterogenous in shapes and sizes, these spines are broadly classified into: Thin,Stubby,Mushroom and Filopodia.Each of them plays a unique and significant role in neural signal transmission. 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. Thin spines are more dynamic than mushroom spines and believed to be "learning spines," responsible for forming new memories during the synaptic plasticity process. Stubby spines typically do not have a neck. They are known to be the predominant type in the early stages of development but are also found in small amounts in adulthood. Filopodia are long, thin dendritic membrane protrusions without a clear head, commonly observed in developing neurons.

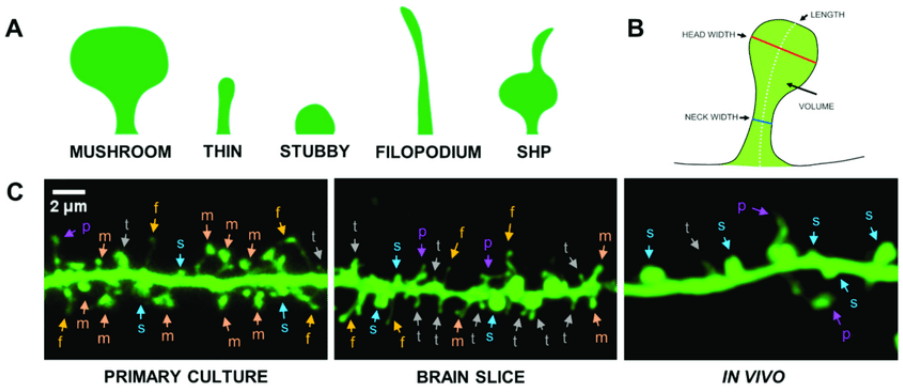


Figure 1.1: Morphological diversity of dendritic spines. (A) Spine shape classification: mushroom (m), thin (t), stubby (s), filopodium (f), SHP-spine head protrusion (p); (B) definition of morphometric parameters; (C) microscopic images of dendrites covered with dendritic spines obtained from in vitro (primary culture), ex vivo (brain slice), and in vivo (cranial window) imaging. Scale bar: 2 m.

However, the spine morphology is dynamic in nature; the structure exhibited by them can change over variable timescales. Alterations in the spine morphology cause an impact on the neural functions as well. Changes in spine density have been observed in response to changes in the efficacy of neurotransmission. Loss of dendritic spines is directly associated with the loss of synaptic function which is an immediate cause of cognitive decline and memory dysfunction in Alzheimer's disease and other neurological disorders. The balance between spine appearance, maturation, elimination, and plasticity is crucial for proper brain function. Methods for examining and understanding the morphology of dendritic spines are critically important for many fields of neuroscience. Our objective is to accurately classify the four types of dendritic spines using CNN. The model is based on fully annotated dataset having 2PLSM images. Once the model's classification results are obtained, the spine density and its associated biological interpretation will be analysed.

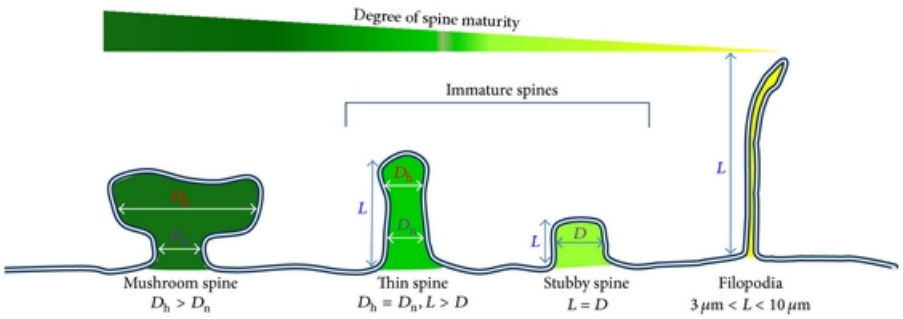


Figure 1.2: Diagram of dendritic spines. Dendritic spines are categorized into mushroom, thin, and stubby spines. Length of spine (L), diameter of spine head (Dh), and diameter of spine neck (Dn)

2 Literature Review

2.1 Neurons

Neurons are information messengers. They use electrical and chemical signals to send information between different areas of the brain, as well as between the brain, the spinal cord, and the entire body. A neuron has three basic parts: a cell body, and two branches called an axon and a dendrite. Within the cell body is a nucleus, which controls the cell's activities and contains the cell's genetic material. The axon looks like a long tail and sends messages from the cell. A dendrite looks like the branch of a tree and receives messages for the cell. Neurons communicate with each other by sending chemicals, called neurotransmitters, across a tiny space called a synapse, between the axons and dendrites of nearby neurons.

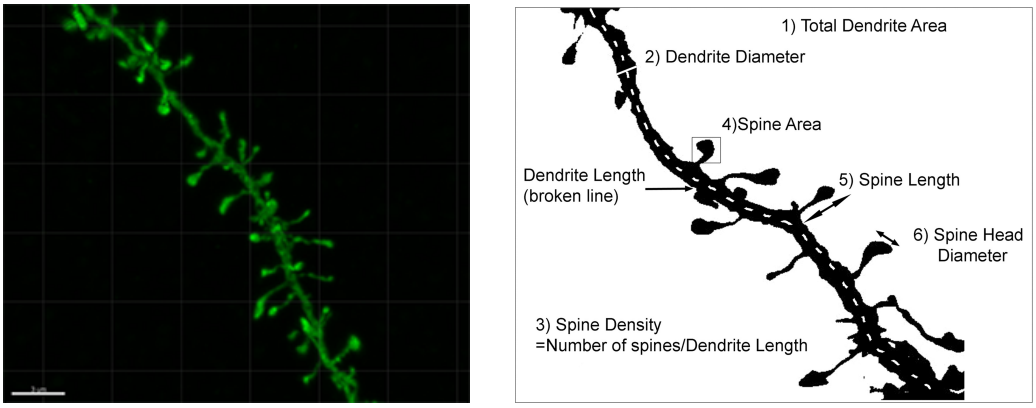


Figure 2.1: A neuron with dendritic spines

2.2 Dendritic Spines

Dendritic spines are small membranous protrusions that exhibit various shapes and sizes [1]. Hence, researchers have been classifying spines based on their morphology to help neuroscientists in identifying different types of spines, understanding brain function and the underlying mechanisms of neurological disorders[2][3]. The segmentation of spines from image data of dendrites is indeed a challenging task since most of these dendritic segments have numerous protrusions[4]. The contour tracing algorithm proposed by Ruszczycki et al., (2012) includes marking two end-points and selecting a suitable segmentation parameter for each spine thus making it a cumbersome method[5][6]. Similarly,the 3-D method proposed by Heck et al. (2014) is not an efficient one as manual supervision and optimisation are needed[7][8]. Subhadip Basu et al. (2016) proposed a 2-D segmentation

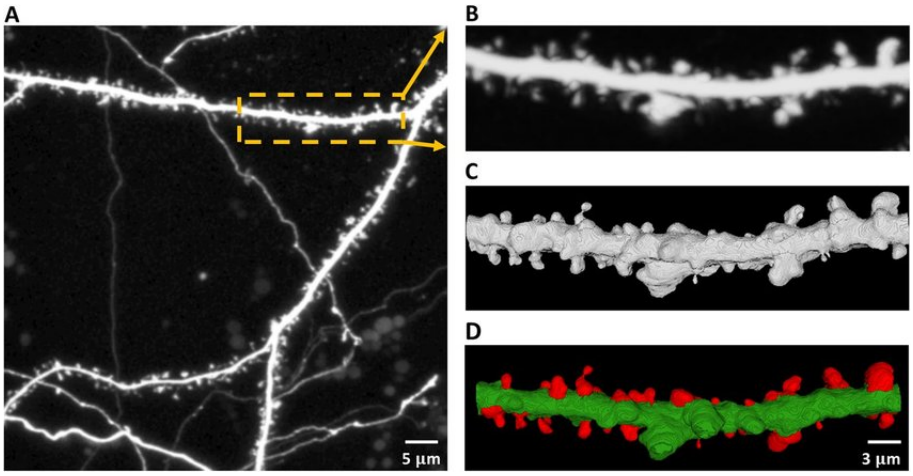


Figure 2.2: Confocal light microscopy image of hippocampal dendrite covered with dendritic spines

method called 2dSpAn that classifies spines utilising convolution kernels .The area, length, neck-length, neck-width,head-width are calculated and estimated following which changes in the measurements are observed after cLTP. One of the limitations primarily include the dependency on binariza-

tion algorithm which may introduce noise in the data leading to incorrect segmentation of spines[9].

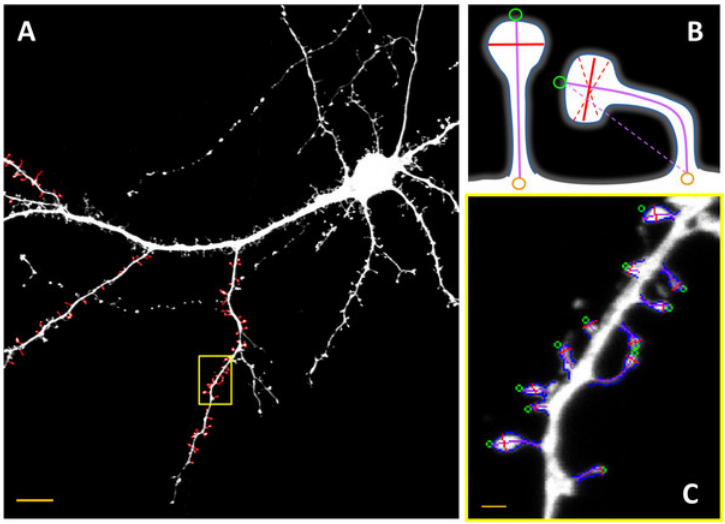


Figure 2.3: (A) Low-power image of neuron with marked spines (red contours) selected for simulations. Only clearly distinguishable transversally protruding spines located on the secondary dendrite were taken. Scale bar is 10m. (B) For each spine we measured the cross sectional area, the head-width and the length. For the symmetric spine there is no ambiguity in definition of the head-width or the length. It is not clear which distance shall represent the spine head-width (dashed red lines). For this reason we used the virtual spine skeleton to measure the length and require the head-width line to be perpendicular to this skeleton. (C) The magnification of a neuron with marked spines selected for simulations (Scale bar: 1m). The recorded parameters were the area (blue contour), the spine length (purple curve) and the head-width (the red line)

Among the existing 3-D approaches, Janoos et al. proposed a 3-D reconstruction based on skeletonization in which the centre of the neuron is extracted, then the longest line is considered as the backbone of the dendrite and the shorter lines are considered as the centerlines of dendritic spines [10]. The entire process is tedious and complex. A recent morphological analysis algorithm for dendritic spines based on semi-supervised learning

was proposed by Shi et al. After the spines are segmented features like head and neck diameter, spine length etc are extracted. Some of the detected spines are chosen for training set and labels are calculated when the detected spines are classified by learning framework.Since the method is dependent on size of training set and features included, the accuracy can be affected[11][12].

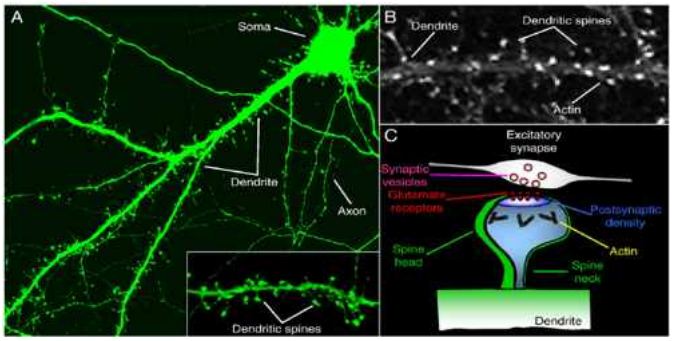


Figure 2.4: Sample of a neuron dendrite

S. Basu, P.K. Saha et al. (2018) introduced a new method of 3-D dendritic spine segmentation using multi-scale opening approach [13]. Imaris tool was utilised in this method which involves segmentation based modelling and feature extraction methodology . However, there is a possibility of corruption of data and this algorithm does not classify spines as thin. Staining quality, image resolution are other factors that hinder the identification of true spine boundaries. The feasibility of using artificial neural networks for spine segmentation was evaluated by Isabel Vidaurre-Gallart , Isabel Fernaud-Espinosa et al.(2022). A ground truth of spine and dendritic shaft reconstructions was built from microscopic images and automatic data preprocessing technique is used. A GUI application has been developed to correct the automatic segmentation and store that to train more accurate models. DL architecture has been used to segment confocal images of dendritic spines [14]. However, it could not solve the limitation of overlapping spines[15].

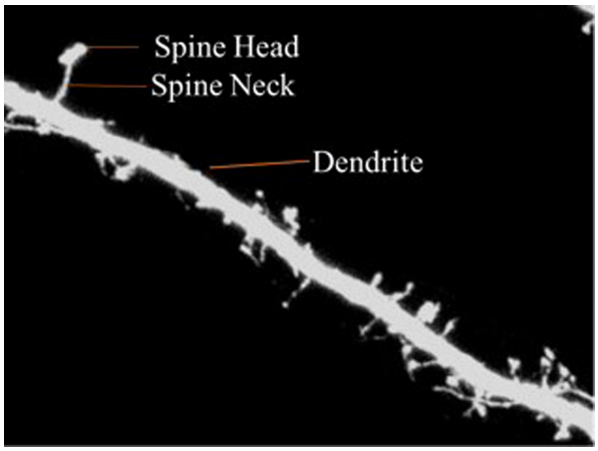


Figure 2.5: Sample of a neuron dendrite

The dendritic spines are minute structures. Hence, distinguishing them from background noise and other neuronal structures becomes extremely difficult. Low resolution or noisy imaging data and non-uniform distribution of the spines can cause even more hindrance when segmentation of dendritic spines are performed. Therefore ,our approach focuses on creating synthetic data in the form of binary images of spine and binarizing pre-existing dataset of spines . This will be followed by training the model using a combination of both real and the synthetic data thus leading to higher accuracy and efficiency.

2.3 Spine Head Detection

Dendritic spine head detection is an important research area in neuroscience due to the critical role of dendritic spines in learning and memory. Dendritic spines are small protrusions from the dendrites of neurons that form the postsynaptic sites of excitatory synapses. The number and morphology of dendritic spines are highly dynamic and can be altered by experience, such as learning and memory formation. As a result, dendritic spine head detection can be used to study how experience shapes the brain and to identify

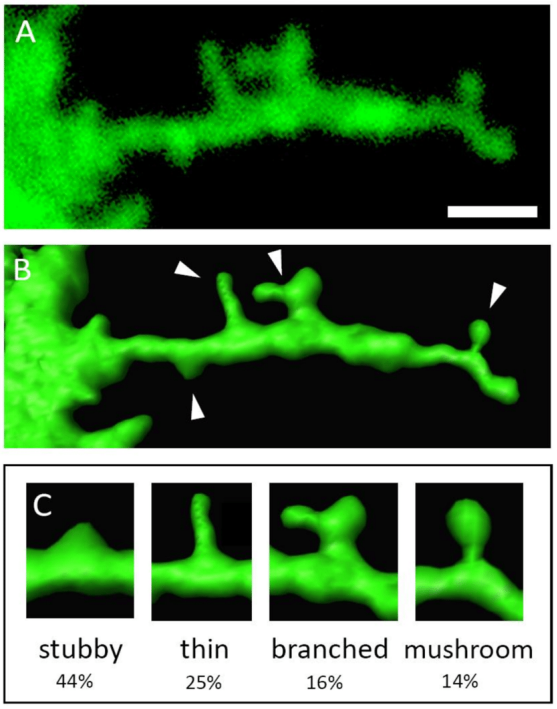


Figure 2.6: Classification of dendritic spines of LPTCs

potential biomarkers for neurological disorders.

- Dendritic spines are thought to be the physical substrate of learning and memory. By studying how the number and morphology of dendritic spines change with experience, we can gain insights into how the brain forms and stores memories.
- Neurological disorders such as Alzheimer's disease, Parkinson's disease, and schizophrenia are often characterized by changes in dendritic spine density and morphology. Dendritic spine head detection can be used to identify these changes and to develop new diagnostic approaches.
- Dendritic spines are considered as one of the key sites of synaptic plasticity. Dendritic spine head detection can be used to study how changes

in dendritic spines contribute to synaptic plasticity.

Challenges faced in spine head detection:

- Dendritic spines are dynamic and can change their morphology quickly.
- Dendritic spines are typically only a few hundred nanometers in diameter, which makes them difficult to image with conventional light microscopy.
- The density of dendritic spines can vary depending on the location of the spine on the dendrite and the type of neuron. This makes it difficult to compare dendritic spine density across different neurons or brain regions.

How are dendritic spine head images captured using a microscope:

Light microscopy is the most widely used technique for imaging dendritic spines. However, the resolution of light microscopy is limited by the diffraction of light, which makes it difficult to image dendritic spines in detail. Electron microscopy can also image dendritic spines in much greater detail than light microscopy. However, electron microscopy cannot be used to image live cells. Therefore, Two-photon microscopy ,the type of light microscopy that uses two photons to excite a fluorophore in the sample is used .This allows us to image deeper into the tissue than conventional light microscopy.

3 Problem Definition

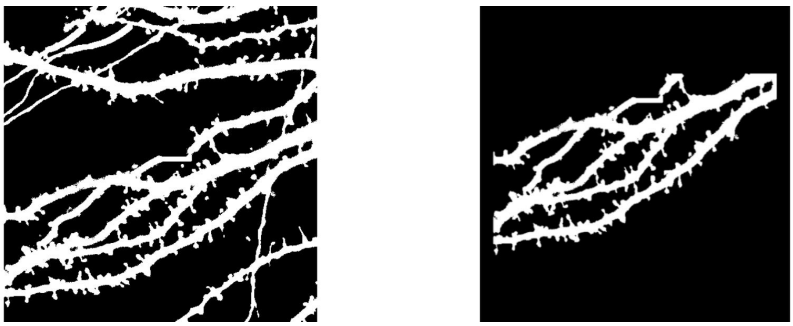
Dendritic spines exhibit different morphological structures and each of them play a unique role in synaptic transmission. Any kind of alterations in the structure can have an impact on neural functions thus making classification of these spines crucial. Based on their morphology, the dendritic spines are

commonly classified into: Thin, Mushroom, Stubby, and Filopodia. The objective of our model is to perform an efficient and accurate classification of dendritic spines using InceptionV3. This model leverages the power of deep learning and the InceptionV3 architecture to classify the dendritic spines into their respective categories based on their morphological features. This classification can provide valuable insights into the structural alterations in dendritic spines and their impact on neural functions.

4 Software Design

In this research endeavour, we undertook a multiclass image classification task to discern four distinct types of dendritic spines, utilizing a two-model approach. The first model, based on the YOLO-NAS architecture, was used for object detection to identify the dendritic spines within larger neuron images. The detected regions were then passed to the second model, a CNN inspired by the InceptionV3 architecture, for classification into one of the four categories: mushroom, thin, stubby, and filopodia. Our exploration was based on a fully annotated dataset of Two-Photon Laser Scanning Microscopy (2PLSM) images, generously provided by a seasoned neuroscience expert. This invaluable dataset encompassed raw data, manual annotations (segmentations), and expert-generated labels, offering comprehensive insights into dendritic spine morphology which is labelled below:

Figure 4.1: Morphological Variations of Dendritic Spines



(a) Sample Images

(b) Mushroom (c) Stubby (d) Thin (e) Filopodia

Figure 4.2: Ground Truth images dataset for each Dendritic Spine Class made from the sample images of spines

Prior to model training, we meticulously pre-processed the dataset by resizing the images to a standard input size and normalizing pixel intensities. Data augmentation techniques were applied to enrich the training set and enhance generalization capabilities. Subsequently, we partitioned the dataset into training, validation, and test subsets. Our CNN model comprised convolutional layers, followed by max-pooling layers, tasked with extracting hierarchical features from the input images. The adoption of ReLU activation and dropout layers helped introduce non-linearity and mitigate overfitting concerns. The output layer employed SoftMax activation to yield probabilities for the four dendritic spine types. The training phase incorporated optimization algorithms, such as Adam, and we monitored the model's performance on the validation set to prevent overfitting. Find the process workflow below:

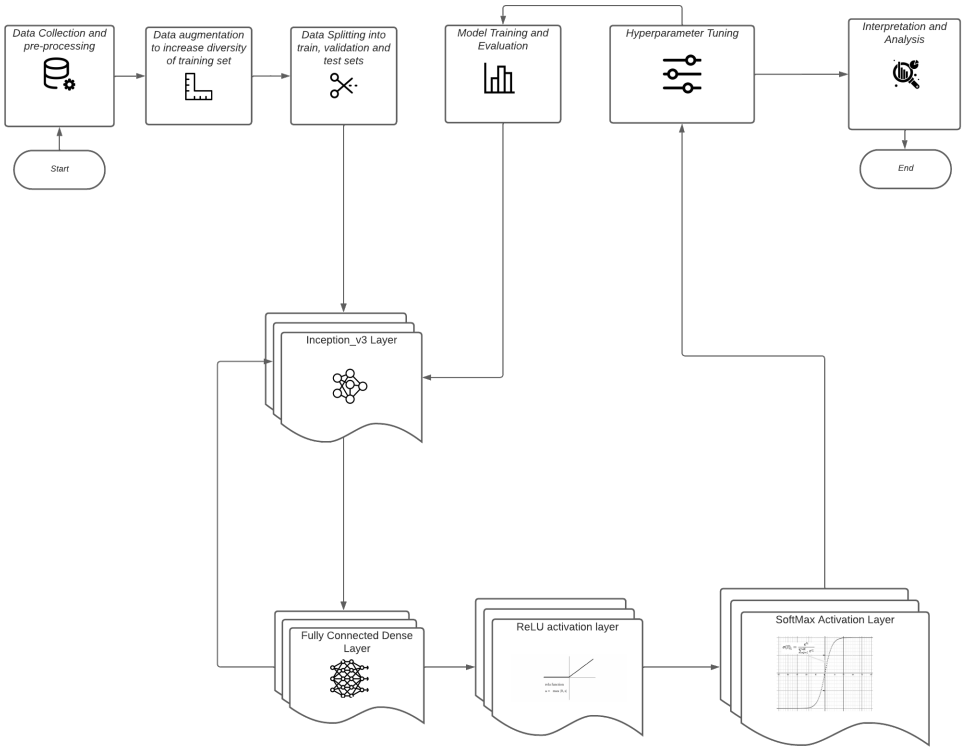


Figure 4.3: Training workflow

To gauge the model's efficacy, we evaluated it on the test set, employing standard multiclass classification metrics like accuracy, precision, recall, and F1-score. The resulting confusion matrix enabled us to discern misclassification patterns and identify potential areas for improvement. Furthermore, we delved into the biological interpretation of the classification results, seeking to understand the implications of the model's predictions in the context of dendritic spine analysis. Throughout this investigation, our primary focus remained on the biological significance of dendritic spine

classification, aiming to contribute novel findings to the field of neuroscience research.

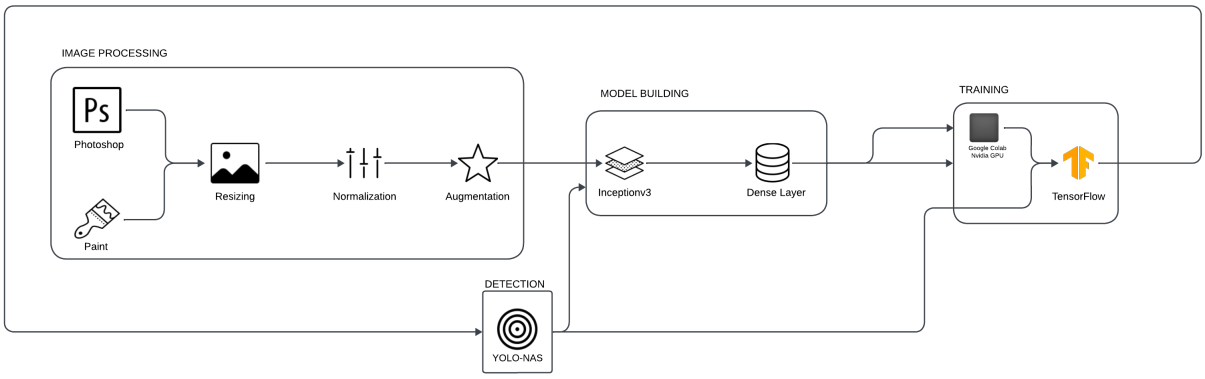


Figure 4.4: Dendritic Spines Analysis Workflow

5 Software and Hardware Requirements

A Nvidia GPU, provided by the free tier of Google Colab will be used for faster training and inference. A GPU having at least 4GB-8GB VRAM is preferred as InceptionV3 is a relatively large model and it has to be trained without any drawbacks. An operating system like Windows, macOS, or Linux that supports Python and deep learning frameworks is required. Python 3.6 (or higher) will be used for compatibility with most of the deep learning frameworks. Necessary GPU drivers for our specific GPU brand (NVIDIA drivers for NVIDIA GPUs) have to be installed. A deep learning framework like TensorFlow, and Keras is needed to support building and training CNN models. Depending on the chosen deep learning framework, the following Deep Learning Libraries will be used: If TensorFlow is used as the back-end, Keras is included in TensorFlow by default. Two additional libraries are required: Matplotlib,a library used for data visualization and NumPy

,which is essential for numerical computing in Python , data manipulation and preprocessing. The InceptionV3 architecture model, obtained from TensorFlow Hub, will be used and modified according to our requirements using the selected deep learning framework.

6 Code Templates

1. **MirroredStrategy:**
    - Functionality: Implements distributed training with multiple GPUs for faster model training.
    - Methods:
        - `tf.distribute.MirroredStrategy()`: Initializes a MirroredStrategy object for synchronous training across multiple GPUs.
        - `mirrored_strategy.scope()`: Creates a scope for the operations within, enabling distribution of computations across GPUs.
2. **Base Model Layer:**
    - Functionality: Creates a base model from a pretrained InceptionV3 instance using TensorFlow Hub.
    - Methods:
        - `hub.KerasLayer(inception_v3, input_shape=(224, 224, 3), trainable=False)`: Initializes a KerasLayer object with the InceptionV3 model from TensorFlow Hub, specifying the input shape and setting the layer to non-trainable.
3. **Dense Layers (x2):**
    - Functionality:

- First Dense Layer (`tf.keras.layers.Dense(1024, activation='relu')`): Applies fully connected layer with 1024 neurons and ReLU activation.
- Second Dense Layer (`tf.keras.layers.Dense(4, activation='softmax')`): Produces the final output with a softmax activation for classification into 4 categories.

- Methods:
  - `tf.keras.layers.Dense(units, activation)`: Adds a fully connected layer with the specified number of units and activation function.

4. **Model:**

- Functionality: Constructs the neural network model using the specified layers and configuration.
- Methods:
  - `tf.keras.Sequential([base_model, dense_layers])`: Creates a model with the specified base model and dense layers.
  - `model.compile(optimizer='adam', loss='categorical_crossentropy', metrics=['accuracy'])`: Compiles the model with the specified optimizer, loss function, and metrics.
  - `model.summary()`: Displays a summary of the model architecture.

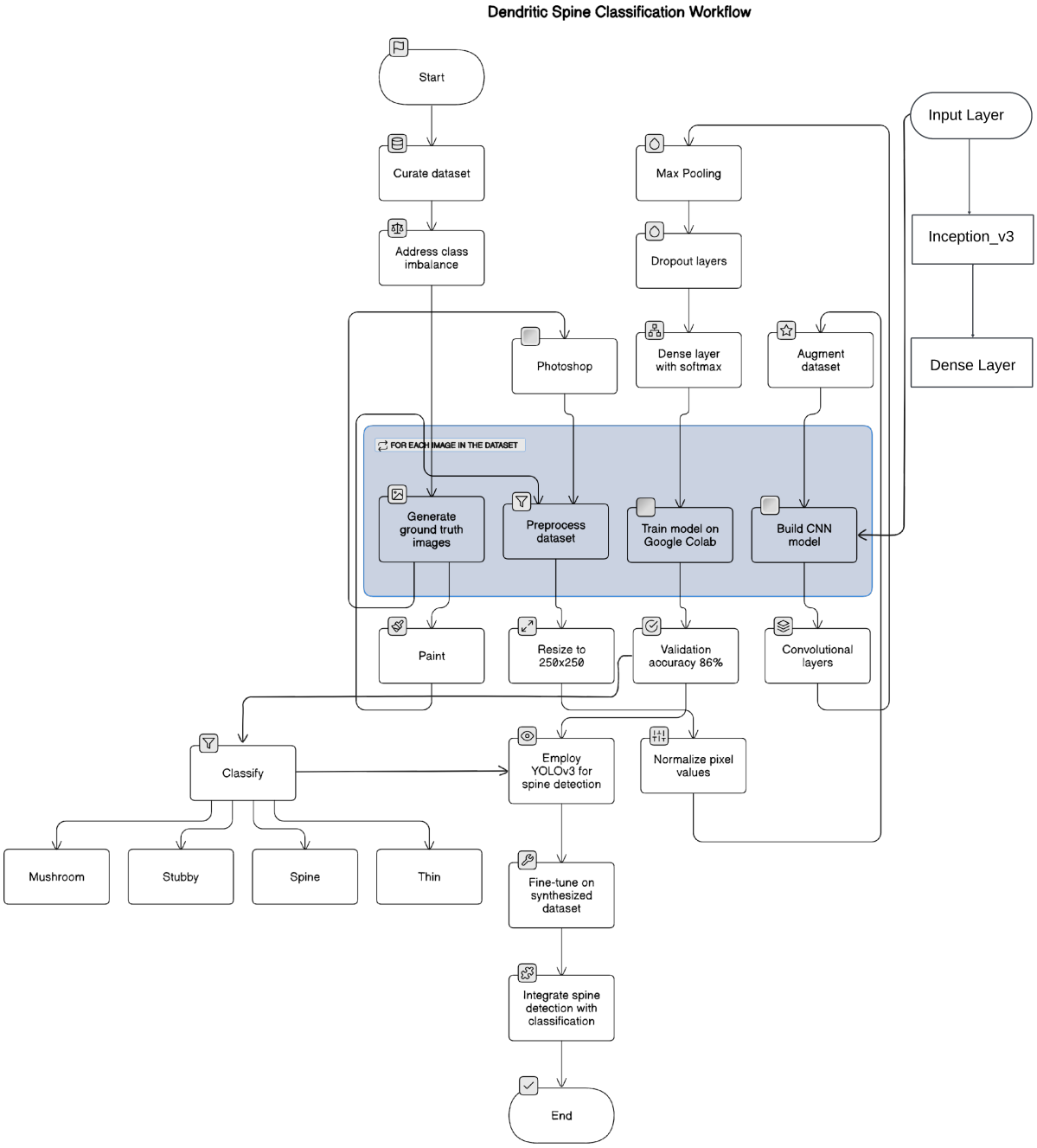


Figure 6.1: Dendritic Spines Classification workflow

This model is designed for classifying dendritic spine shapes into Mushroom, Stubby, Thin, and Filopodia categories, with the softmax activation in the last dense layer providing class probabilities. The model architecture uses a pre-trained InceptionV3 model as a base model, followed by dense layers for classification. The MirroredStrategy enables efficient distributed training across multiple GPUs.

7 Experimental Results and Analysis

Our experimental results demonstrated the efficacy of our two-model approach in classifying dendritic spines. The YOLO-NAS model successfully detected the dendritic spines within the larger neuron images, and these detected regions were then classified using the InceptionV3 model.

The InceptionV3 model achieved an impressive accuracy of 91.07% on the test set. The accuracy curve graph, which plots the model's accuracy over the course of training along with the The loss curve graph, which shows a decrease in loss over time, is shown below:

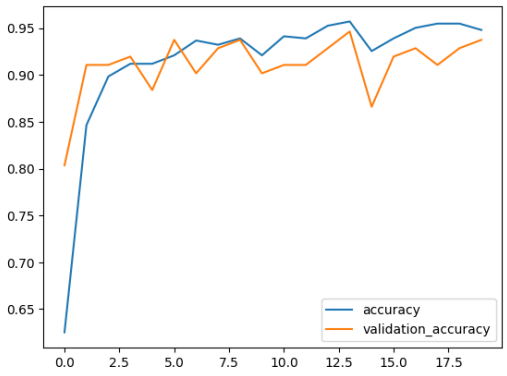


Figure 7.1: Accuracy Curve

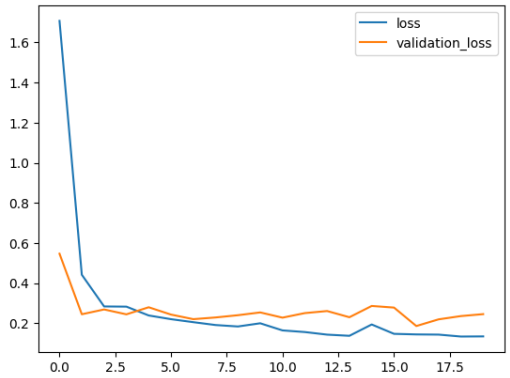


Figure 7.2: Loss Curve

The ROI classified images provide a visual representation of the model's performance. In these images, the detected dendritic spines are highlighted and correctly classified. An example of these images is shown below:

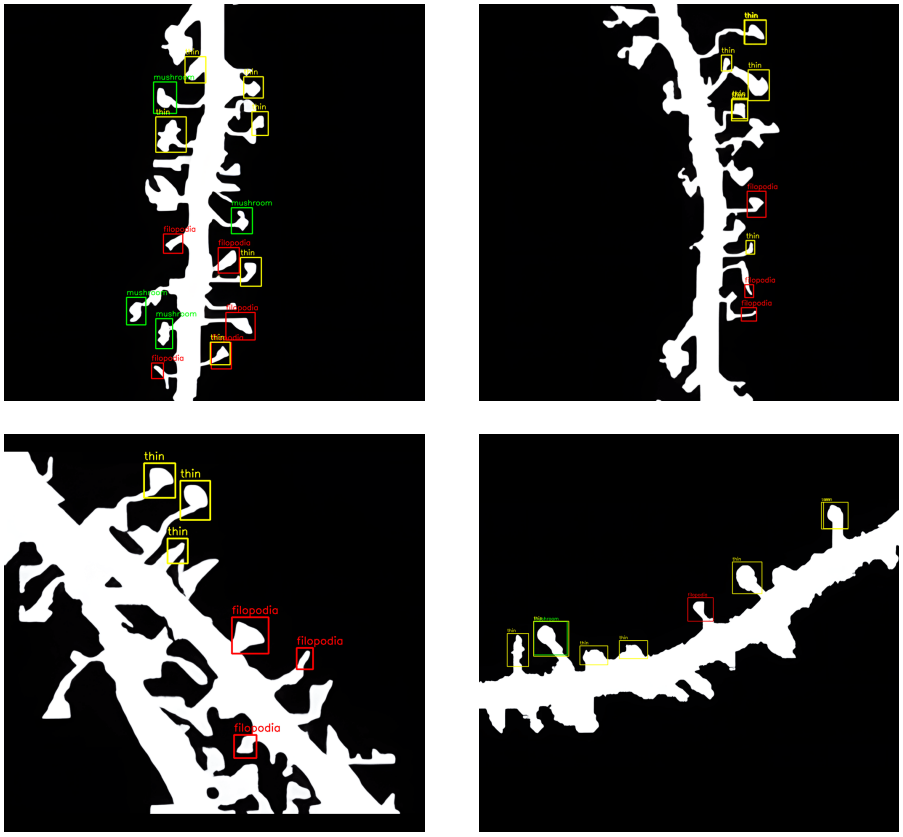


Figure 7.3: ROI Classified Images

These results demonstrate the effectiveness of our approach in classifying dendritic spines. Future work could explore the use of other state-of-the-art models or the incorporation of additional features to further improve the model's performance.

8 Conclusion

This research has successfully developed a machine learning model capable of accurately classifying dendritic spines into four distinct types: mushroom, stubby, thin, and filopodia. The model's performance has been meticulously evaluated, demonstrating its robustness and reliability in spine classification. The use of synthetic ground truth images and comprehensive preprocessing techniques has significantly contributed to the model's performance, addressing class imbalance and enhancing the suitability of the dataset for model training.

The integration of the **YOLO-NAS object detection model** with the **InceptionV3-based convolutional neural network (CNN) model** forms a unified pipeline for spine detection and classification. This innovative approach showcases the adaptability of our methodology to dendritic spine analysis. The pipeline first detects the dendritic spines within larger neuron images using YOLO-NAS, and these detected regions are then classified using the InceptionV3 model. This pipeline is expected to be a valuable tool for neuroscientists, aiding in the investigation of the role of dendritic spines in various neurological disorders.

Future work will focus on further improving the accuracy of the model and expanding its capabilities to include more spine types. Additionally, the integration of the model with other imaging techniques will be explored to enhance its applicability in real-world scenarios. The potential for combining other state-of-the-art models for object detection and classification will also be considered, aiming to further enhance the performance and versatility of the pipeline.

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