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Senior Thesis

Neural Network Mitosis Cell Segmentation

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Abstract

The applications of artificial intelligence (A.I) are endless and soon it will become a part of everyday life. As A.I continues to improve, research in a variety of fields will become more efficient due to computers making specific tasks, that were once tedious, easy to do in a short amount of time. A promising aspect of A.I is known as neural networks which is a method that teaches computers to process data in a way that is inspired by the human brain. This process uses interconnected nodes or neurons in a layered structure that inputs pass through, that help the computer make a prediction as the output. Moreover, it is an adaptive system that computers use to learn from their mistakes and improve continuously. This plays a role in why this aspect of A.I is so powerful because with time and more inputs, it can become more accurate in making predictions. Knowing how useful this tool is, how can A.I be used for research in cellular biology? In this research paper, we explored the use of neural networks in the determination of the stages of mitosis based on images of cells. This process is known as neural network image classification, and it will be used to classify images of cells undergoing mitosis. Five different cells were imaged separately in a time-lapse fashion to capture the totality of the mitosis life cycle. These images were then processed by InceptionV3, a pretrained neural network that was specifically used for image classification. Finally, the neural network made predictions on what stage of mitosis each image was in. Our research found that the implemented neural network was capable of accurately determining the stage of mitosis that different images of cell were in. Additionally, it was determined that different data augmentation techniques can be used to increase the accuracy of the neural network. These findings support the idea that A.I can be a useful tool for a variety of research application and can aid researchers in making new discoveries. As aforementioned, a current challenge to mitosis cell segmentation is the tedious task of manually looking through images and separating them into the different mitotic stages. This challenge becomes more difficult, especially when looking at images in between stages. Our neural network model provides a more mathematical and comprehensive way to determine the mitotic stages of cells that leaves less room for human error.

Introduction

Artificial intelligence (A.I) has been an area of interest for researchers in various fields for many years. As A.I technology advances, it is becoming increasingly important to explore its potential applications in different areas, including cellular biology. In particular, neural networks, a powerful method that teaches computers to process data in a way that is inspired by the human brain, have shown great promise in the field of image classification. Mitosis, the process by which cells divide and produce identical daughter cells, is a crucial aspect of cellular biology that has been extensively studied for many years. However, determining the stages of mitosis from images of cells can be a tedious and time-consuming task that requires expertise and careful observation. A.I, and more specifically neural networks, have the potential to aid in this process by automating the classification of mitotic stages in images of cells.

In this research paper, we aim to explore the use of neural networks in the determination of mitotic stages based on images of cells. We will use a neural network image classification approach to classify images of cells undergoing mitosis into different stages. Our study is based on the use of the InceptionV3 neural network, which was pre-trained for image classification. We will investigate the accuracy of the neural network in predicting the mitotic stages of cells and explore the use of different data augmentation techniques to increase its accuracy.

The potential applications of this research are significant. Automated image classification of mitotic stages in cells can aid in the understanding of cell division and cellular biology. It can also be used to screen for abnormalities in cell division, which can be crucial in the diagnosis and treatment of cancer and other diseases. Additionally, this research has the potential to save time and resources for researchers in the field of cellular biology, who often spend countless hours manually classifying images of cells. In summary, this research aims to explore the use of neural networks in the determination of mitotic stages based on images of cells. By automating the classification process, this research has the potential to aid in the understanding of cellular biology and save time and resources for researchers in the field.

Methods

In order to train our neural network, the data had to be prepared and organized in such a way that the neural network could interpret. The first part of the process was obtaining our images. Phase contrast imaging was performed on 5 separate cells without fluorescence. This is the classic approach to imaging mitosis because it allows for the imaging of cells that lack fluorescent markers (Grah et al., 2017). Images were taken as a time lapse to help capture the entirety of mitosis along with the different aspects between and within each stage. A total of 3115 images were taken of 5 different cells undergoing mitosis (Figure 1.)

After phase contrast imaging was completed, the images were separated based on what stage of mitosis the image corresponded with. The stages of mitosis that were taken into consideration were condensation, prometaphase, metaphase, and anaphase. Separation of the images into the different stages of mitosis occurred in a systematic fashion. Because the images were taken as a time-lapse, groups of images could be separated into different stages after a key aspect of a particular stage was clearly found. The end of condensation was marked by the disintegration of nucleolus meaning that images before this point were considered to be in condensation. Images placed in the prometaphase group were mainly delineated as the images after the breakdown of the nucleolus and before the alignment of sister chromatids along the metaphase plate. Images placed in the metaphase group were the images after the alignment of sister chromatids along the metaphase plate and before the separation of chromosomes to opposite poles of the cell. Finally, images placed in the anaphase group were the images that showed sister chromatids being pulled to opposite poles of the cell. Additionally, images in between stages that could not be determined to be in one mitotic stage or another were placed in a separate grouping of images known as buffers. For example, the group of images within the prometaphase buffer represent the images that could not be clearly determined as condensation or prometaphase. Furthermore, the group of images within the metaphase buffer group represent the images that could not be clearly determined as prometaphase or metaphase, likewise for the anaphase buffer group.

Once each image was labeled in association with a particular mitotic stage, a subset of images from each cell was placed in a training set, validation set, and a test set. The training set is the group of images that the neural network will be trained on. The buffer images were not included in the group of images that the model would be trained on. This is because the images

used to train a neural network should be clear examples of each mitotic stage, thus allowing the neural network to unambiguously learn and determine the unique characteristics of each stage. When a neural network runs, it goes through multiple rounds of learning known as epochs. Within each epoch, the neural network gets trained on the training set, learning from the mistakes made in the previous epoch. Once training has been completed it will then try to make predictions about the validation set, a different group of images that the neural network has not encountered, that is used to evaluate the performance of the neural network during training. The test set is also a different group of images that the neural network has also not come across and will be used to evaluate the final performance of the model. This set of images does not come into play until the end of the research.

The training set, validation set, and test set all have groups of images that correspond with each stage of mitosis, however the total amount of images within each set differs from one another. In the initial conditions of our datasets, the training set had 410 total images, the validation set had 192 total images, and the test set and 100 total images. The number of images in the test set will remain constant throughout the entirety of the research. After the training, validation, and test sets were made the next step of the process was to code/implement inceptionV3, a pretrained neural network, into google collab. In addition, code in python was added to visualize the performance of the model over time and to create an interface that would allow the user to upload images of cells into the neural network to determine the network's prediction of what mitotic stage the image was in.

Once the neural network interface was constructed, the following stage of the process was to determine the correct number of epochs that the neural network should run for. As aforementioned, an epoch refers to when the entire training set is passed through forward and backward through the neural network one time (Sharma, 2019). More than one epoch must be used because passing the entire training set through a neural network once is not enough for the neural network to learn from its mistakes. In contrast, using too many epochs would be redundant because after a certain number of epochs the neural network could not become any more accurate. To determine the number of epochs to use the initial conditions of our data were passed through the neural starting with an arbitrary 10 epochs. The number of epochs used then increased from 10 to 25, and then to 35. The correct amount to implement was established by determining at what point the training accuracy of the model plateaued. After 15 epochs the

training accuracy of the model no longer improved and going forward this will be the amount used for the neural network (Figure 2).

The final portion of the research was dedicated to continuously improving the neural network's ability to accurately determine the mitotic stage of cells, by changing the conditions that the neural network was trained under. The initial conditions are as aforementioned, and 15 epochs will be used with all subsequent conditions. The initial conditions will be known as condition A. The next set of conditions (condition B) is training the neural network with as many images as reasonably possible. In this condition, 1751 total images were contained in our training set and 554 total images in our validation set. Condition C is characterized by a training set with 1550 cropped images, that eliminate background noise, and contains a more equal distribution of image in the 4 mitotic stages (Figure 3). Condition D has 1048 cropped images in the training set and 187 images in the validation set. Moreover, a stark difference between condition D and the previous conditions is that the images in the training set have been relabeled such that the images that were labeled condensation and prometaphase have been joined together and labeled as prophase. The means that the neural network has only 3 predictions (prophase, metaphase, and anaphase) to make rather than 4. Another significant difference is that the images in the training set of condition D are the highest quality and clearest examples of each stage of mitosis out of our total batch of images. Condition D also has an equal distribution of images in each stage such that roughly 33% of images lie in prophase, 33% lie in metaphase, and 33% lie in anaphase. In the final condition (condition E), the neural network was trained under the same conditions as condition D, however code was implemented such that the neural network would randomly receive images in the training set that were horizontally flipped, vertically flipped, or both to pass through the neural network. After each condition was passed through the neural network, graphs visualizing the training accuracy, validation accuracy, training loss, and validation loss were constructed and examined to assess the capability of the neural network.

Results

Condition A, being the first run through the neural network, was unable to accurately determine mitotic stages. The neural network during this condition was able to reach a training accuracy of 91%, however the validation accuracy hovered consistently around 30%. On top of that, the training and validation loss data indicated that extreme overfitting of the model occurred, further contributing to the conclusion that the neural network at this stage was inaccurate and not useful (Figure 4). Condition B had similar results to that of condition A. This version of neural network reached a training accuracy of 91%, but also maintained a validation accuracy around 30%. However, the overfitting was far less extreme. The overfitting in condition B was reached a peak of 12 which was far better than Condition A whose peak was 40 (Figure 5). The significance of the results of condition B is that the neural network is not able to accurately determine the mitotic stages of cells. Condition C resulted in a 10% improvement in validation accuracy while maintaining the same peak training accuracy as the previous conditions of the neural network. In spite of that, the extent of overfitting and peak training accuracy matched that of Condition B (Figure 6). The improvement in validation accuracy suggests that cropping the images and having a more equitable distribution of images of cells from each mitotic stage can help to improve the model. However, the capability of this rendition of the neural network remained limited in its ability to achieve the research goal. After making many notable changes from condition C to condition D, the next iteration of the neural resulted in a vastly improved model. The training accuracy reached a peak of 93% and the validation accuracy reached a peak of 90%. Moreover, the overfitting tremendously decreased as well. Relative to condition A the overfitting decreased by 98.4% (Figure 7). Finally, condition E continued the trend on improvement. The final version of the model had a peak training accuracy and validation accuracy equal to that of condition D, but the overfitting relative to that of Condition D decreased by 40% (Figure 8). To test the accuracy of condition E the test set was used to determine how well the model works when exposed to images it had never seen before. The test set was composed of 100 images of cells of known mitotic stages from the same cells that the model was trained on. When passing the test set through the neural network, it was able to accurately determine 85 out of the 100 images of the test set.

Conclusion

The results provide information about how to properly train a neural network. Neural networks do a great job of training itself from the training set, however the method the network goes about doing so is critical to the overall accuracy of the neural network when new images are introduced. Condition A, condition B, and condition C exemplify neural networks that were inadequately trained relative to conditions D and E. During condition A the neural network was trained on a training set that contained some low-quality images that were not clearest example of a particular phase of mitosis. The thought process behind the changes made from condition A to B was to incorporate as many photos as possible, therefore allowing the neural network to better generalize the stage of mitosis. Consistent between these two conditions was the integration of low-quality images. The results of condition A, B, and C show us that even though the neural network can train itself well on the training set data, when introduced to the validation set the neural network cannot accurately determine the mitotic stages of a cell. This is validated by the high training accuracy and low validation accuracy seen in figures 4, 5, and 6. A reason that a neural network can have high training accuracy and low validation accuracy is due to overfitting. An often-encountered issue in deep learning is when a model attempts to perfectly fit the training data, leading it to memorize the patterns and even the noise and arbitrary variations present in the data. Overfitting can be seen when the validation loss gradually supersedes the training loss (Baheti, P. 2021). Neural networks use a loss function which is a mathematical function that measures the difference between the predicted output of a model and the actual output. Training loss refers to the value of the loss function on the training set and validation loss is the value of the loss function on the validation set during training. In general, the goal of the neural network is to minimize both training and validation loss, while avoiding overfitting to create a neural network that performs well on new, unseen data.

When comparing the results of conditions A, B, and C positive takeaways for how to train a neural network can be gathered. Though these versions of the neural network did not achieve the research goal, cropping photos, an even distribution of images from different mitotic stages, and having a large number of photos improved the accuracy of the neural network. Decreasing the background noise and environment allows the neural network to focus on more important aspects of different mitotic stages. Additionally, the use of higher quality images limits the potential for contradictory images to be a part of the training set. As aforementioned, a common problem

neural networks have is memorizing the pattern of the training set rather than the actual aspects of the images. For example, let say a neural network is trained from a training set with 90% of the images labeled as metaphase. When this same neural network encounters an image it has never seen before and is unsure about which stage of mitosis the image stems from, it is more inclined to predict that the image represents metaphase (Gyori et al., 2021). This may explain why we see a 10% increase in validation accuracy seen in condition 3 (Figure 6).

Taking these things into consideration, conditions D and E showed significant improvements. A significant factor causing the improvement is the fact that the neural network went from having to predict condensation, prometaphase, metaphase, and anaphase to only prophase, metaphase, and anaphase. This makes the job of the neural network inherently easier because the possible choices to choose from have decreased. This also plays a role as to why these versions of the neural network resulted in significantly less overfitting. Despite this, when testing the neural network on the test set, conditions D and E do a good job of predicting the phase of mitosis. One of the more important reasons for why this may be the case is selecting the highest quality of images to train the neural network on rather than quantity. After looking at the training sets of the previous conditions it was evident that some images were too distorted and ambiguous as to what stage of mitosis the corresponded with individually. In an ideal world, millions of high-quality images would be used to train the neural network on, but those conditions are less viable. The prioritization of quality over quantity in the network was paramount to the success of the model. The images in the training sets of conditions D and E were carefully chosen to ensure that blurry images were not included. Furthermore, the pictures chosen were less ambiguous than previous conditions. Lastly, another factor that contributed to the accuracy of the neural network is data augmentation. Data augmentation can be used to train the data on images that are slightly different in some way to increase the generalizability of the model. For example, training the data on images that are possibly upside down or horizontally flipped can help the neural network understand important patterns to take into consideration. Though it did not improve the network significantly, it did decrease the overfitting within the model.

In conclusion, the results highlight the importance of properly training a neural network to achieve high accuracy in the classification of images. The use of high-quality images, even distribution of images from different classes, and careful selection of images are crucial factors to consider in the training process. Overfitting can be a challenge in deep learning and can be

avoided by minimizing the difference between the training and validation loss through a variety of methods. Additionally, reducing the number of classes in the classification task can simplify the job of the neural network, resulting in better accuracy. Moreover, data augmentation is a useful technique that can help improve the generalizability of the model. Overall, these findings provide valuable insights into the development of effective strategies for training neural networks, which can have important applications in a wide range of fields, including image classification, object recognition, and natural language processing.

Discussion

Though the neural network did a good job of making predictions as seen from the test set results, there are some limitations within the research that prevent the neural network being able to make predictions on all cells. Training sets containing only a few thousand images are not enough to train a neural network to make accurate predictions on all types of cells. In order to make a neural network that is broader based, the amount of quality images would have increase by several orders of magnitude. Additionally, more cell lines within the training set would be necessary to account for the different shapes and sizes that cells can take on. These adjustments would help the neural network become more accurate especially with cell lines it has not encountered. Another limitation of the research was the categorization of images within the training set as prophase, metaphase, and anaphase. Though there was a systematic process to decide which images corresponded with which phase, it is one that is relatively subjective and miscellaneous at times. Subjectivity can lead to neural networks that are very biased and reflect one's personal decision making. In order to prevent this, a more comprehensive method is necessary to determine what stage of mitosis an image reflects. A potential method to do so, is to measure gene expression at the same time that cell imaging is taking place. Afterwards, one could match images to different stages of mitosis based on what genes are highly expressed during the cell imaging process. The expectation that a neural network could be omnipotent in determining the stage of mitosis is improbable. However, to some extent, all models are wrong, but some are useful. Creating a useful model is not far-fetched and with some adjustments it can be used to aid research in the future.

Future directions of this research include looking into different conditions that may lead to a more accurate neural network. The architecture of the neural network remained constant

throughout the process and making changes to the structure may be helpful in increasing the overall performance of the neural network. Another possible direction is to construct/implement multiple neural networks in order to make predictions on specific aspects of the cell. This method would incorporate the results of the different neural networks to make predictions on mitotic stages. This method could be more accurate because it would ensure that specific aspects of each stage would be considered by each neural network used. If an accurate model is constructed that can determine different mitotic stages from images of cells there is potential for a live interface to be developed that can make predictions of the mitotic stages of cells in real time. This would involve overlaying a live feed to a neural network that had object detection capability along with the ability to segment mitotic stages. A system like this would tremendously aid researchers studying a variety of fields such as cancer research and surgery. The utilization of such a program may be crucial for making new discoveries that can progress science as a whole.

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Figures/Table

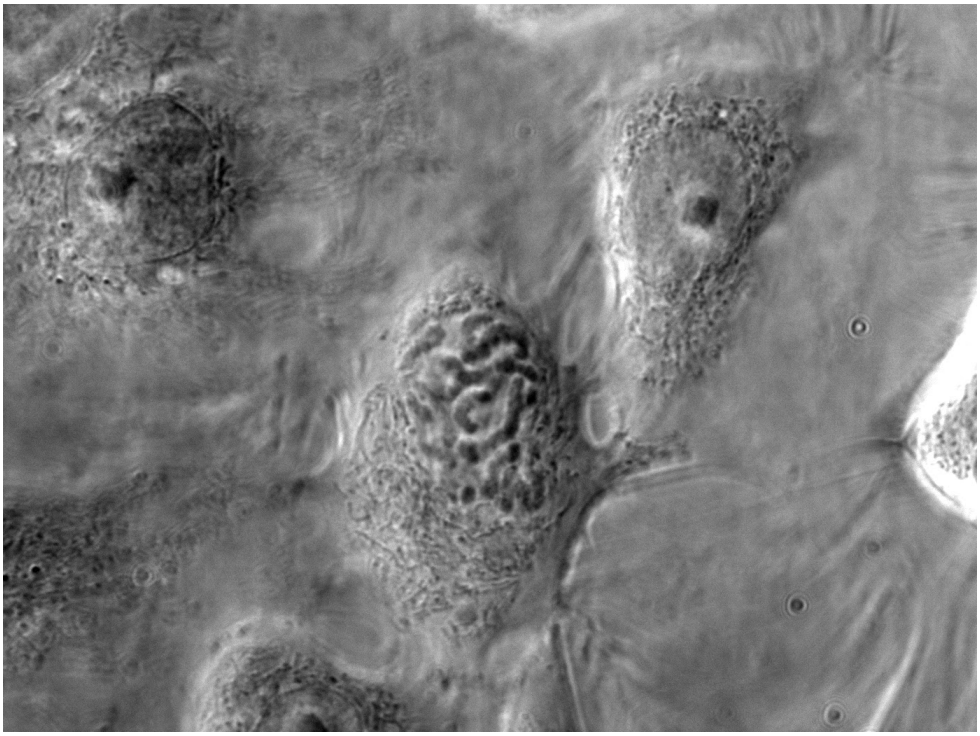

Figure 1. Original phase contrast image of a cell undergoing mitosis. The cell in the middle of the image is the cell of interest. These images were used in Condition A and B.

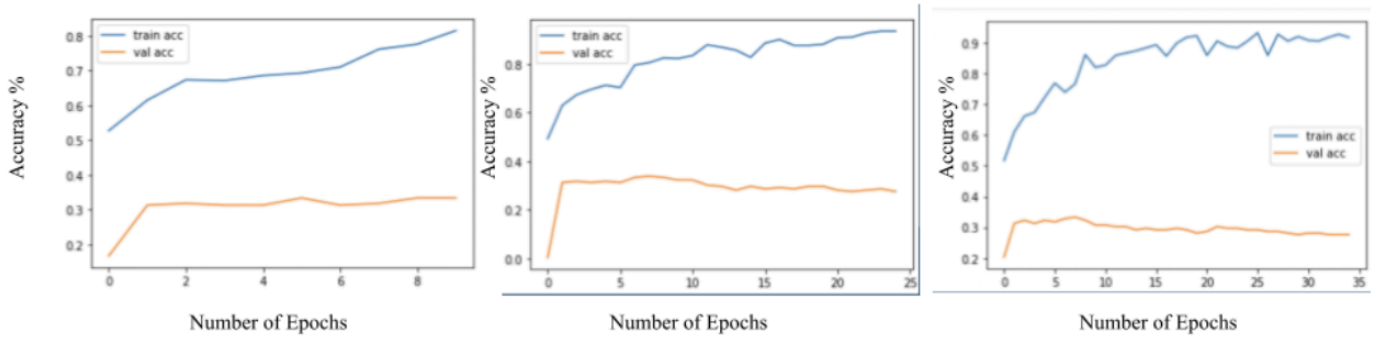

Figure 2. Training Accuracy (blue) vs. Number of Epochs

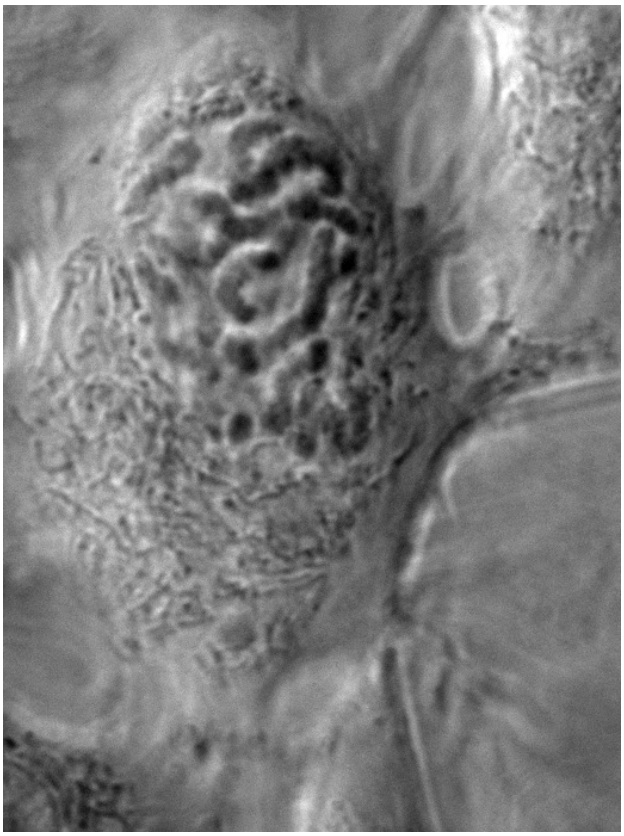

Figure 3. Cropped phase contrast image used in Condition C, D, and E

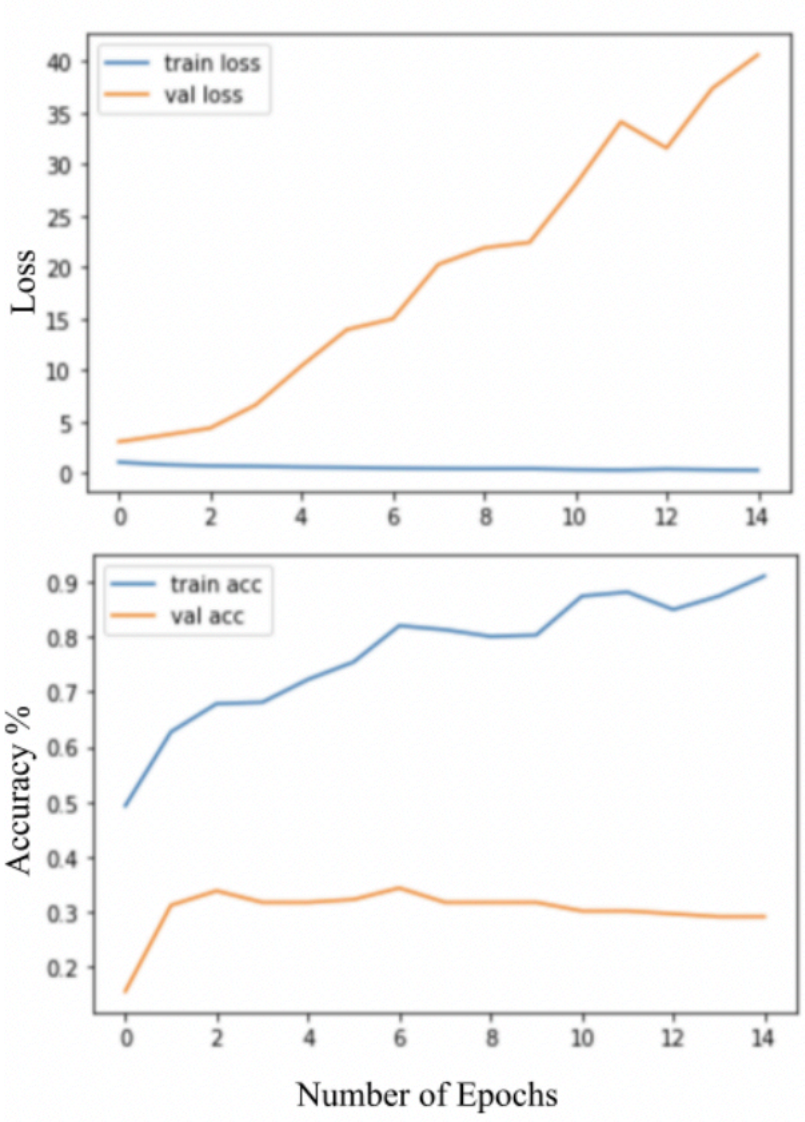

Figure 4. Condition A: Accuracy and Loss results

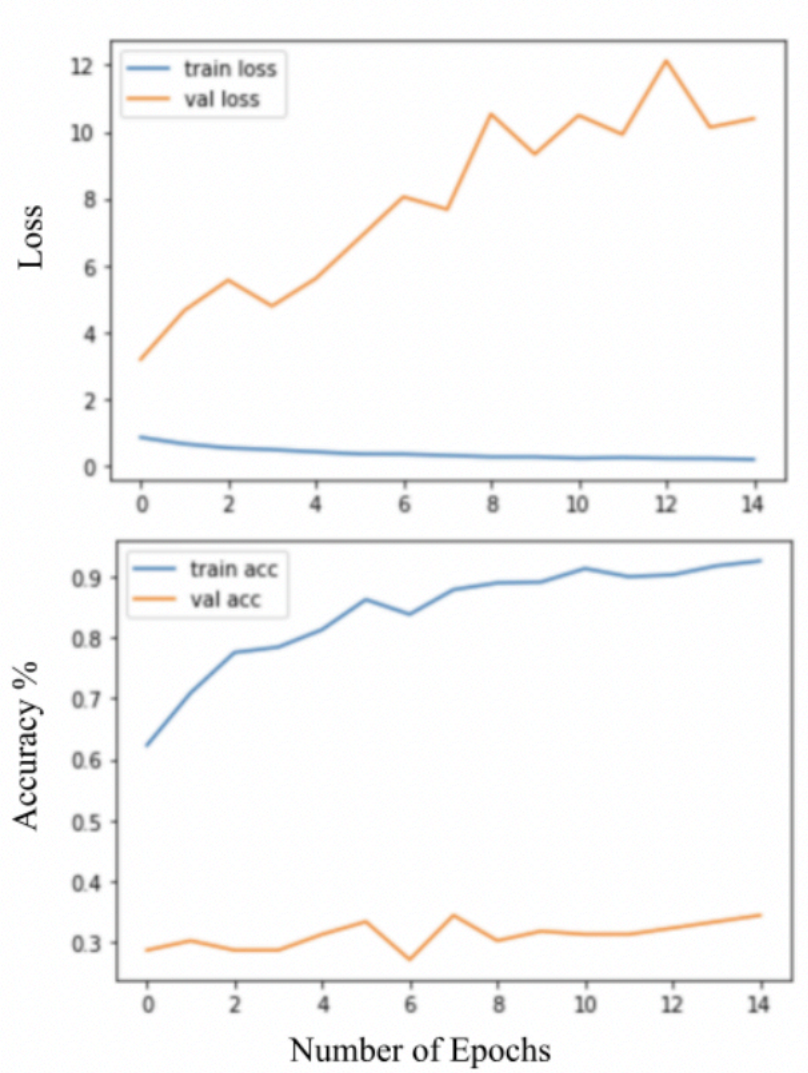

Figure 5. Condition B: Accuracy and Loss results

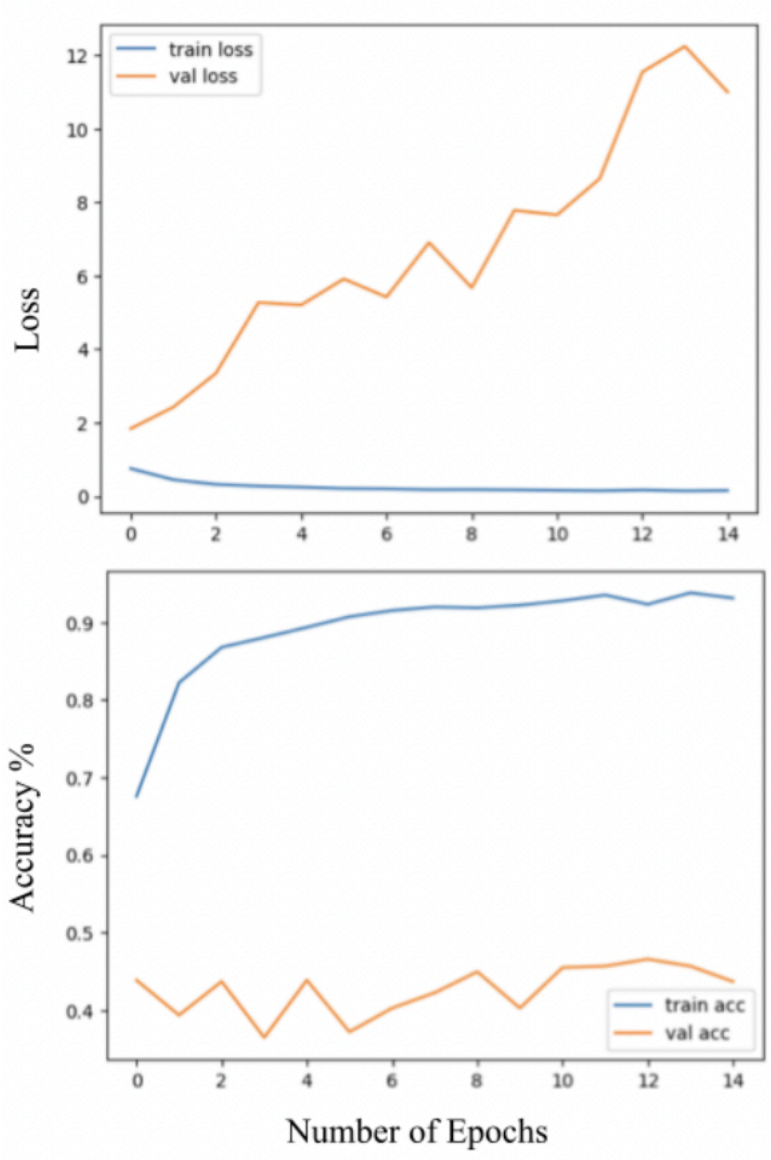

Figure 6. Condition C: Accuracy and Loss results

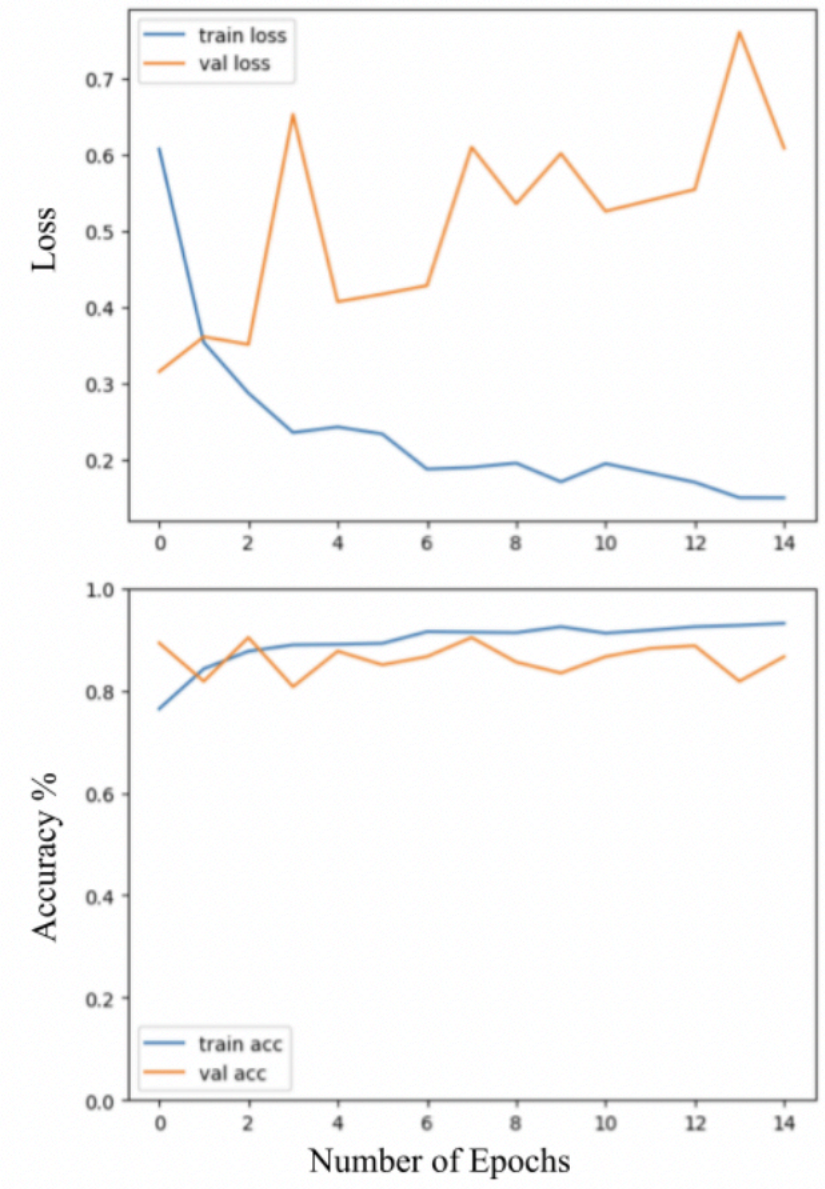

Figure 7. Condition D: Accuracy and Loss results

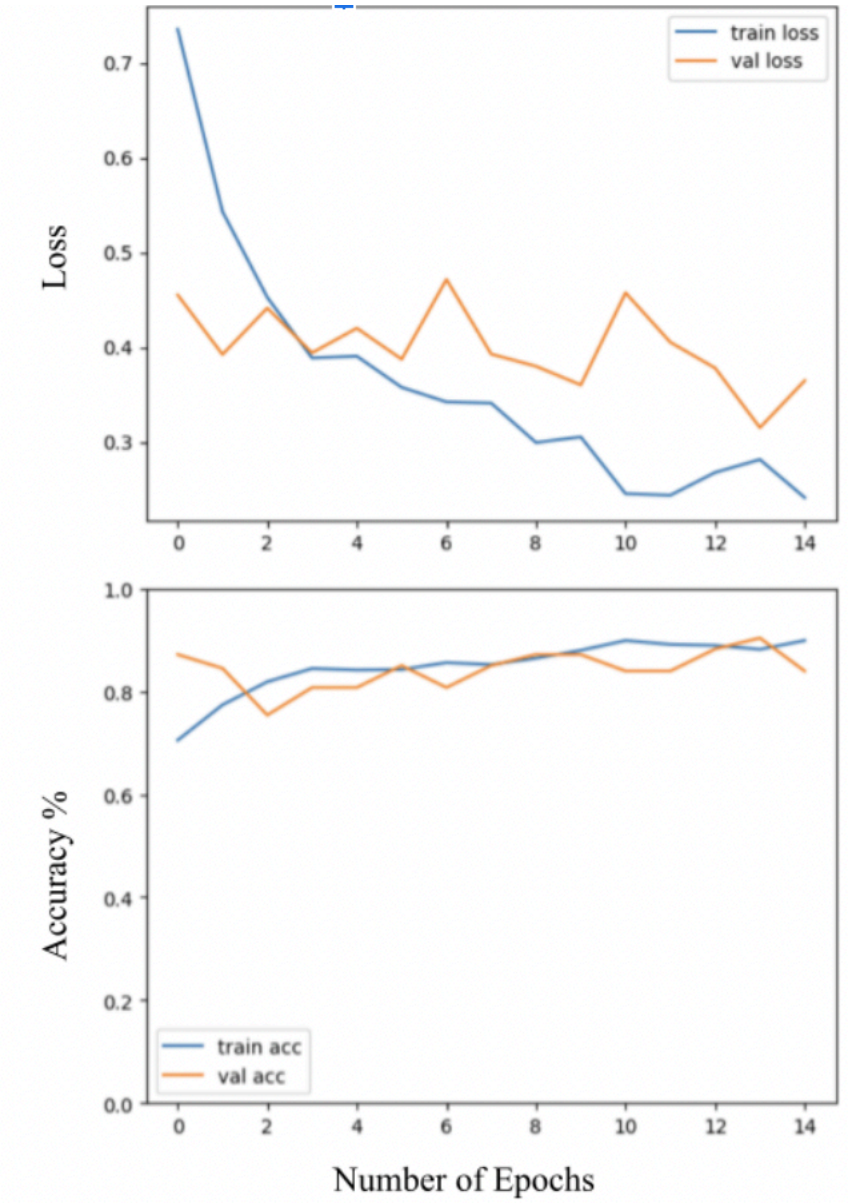

Figure 8. Condition E: Accuracy and Loss results