# PREDICTIVE NEURAL STEM CELL DIFFERENTIATION USING SINGLE-CELL IMAGES BASED ON DEEP LEARNING

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***Abstract*** – The process of neural stem cell (NSC) differentiation into neurons is crucial for the development of potential cell-centered treatments for central nervous system disorders. However, predicting, identifying, and anticipating this differentiation is complex. In this study, we propose the implementation of a convolutional neural network model for the predictable recognition of NSC fate, utilizing single-cell brightfield images. The results demonstrate the model's effectiveness in predicting NSC differentiation into astrocytes, neurons, and oligodendrocytes, achieving an accuracy rate of 91.27%, 93.69%, and 93.06%, respectively. Moreover, our proposed model effectively distinguishes between various cell types even within the initial day of culture.

***Keywords***—Neural stem cell differentiation, Convolution neural network, Single-cell images, Stem cells, Deep learning.

## I. INTRODUCTION

Stem cells represent a specialized cell category with the capacity to differentiate into various distinct cell types, thus playing a pivotal role in the development, maintenance, and regeneration of tissues and organs [1, 2]. The abilities of stem cells to self-renew and form different mature cells expand the possibilities of applications in cell-based therapies in regenerative medicine such as recomposing tissue, drug screening, and treatment of neurodegenerative diseases [3]. Besides, their therapeutic effects result from the secretion of trophic tissue factors, as well from as interactions with infiltrating cells of the immune system through soluble molecules and exosomes [4, 5]. In the adult mammalian central nervous system (CNS), neurogenesis occurs in two specific areas: the subventricular zone and the dentate gyrus found within the hippocampus. Within these regions, the production of various neural cell types is initiated from adult neural stem cells (NSCs). The evaluation of NSCs as a therapeutic approach for addressing CNS diseases and injuries has been ongoing for decades. Parkinson's disease, in particular, has gained the greatest momentum for potential therapeutic benefits [5].

NSC can self-renew or differentiate into neurons and glial cells (astrocytes, oligodendrocytes, and microglia) [1, 6]:

- Neurons: Neurons are fundamental cells responsible for transmitting information in the nervous system, communicating through electrical and chemical signals, using axons to send signals and dendrites to receive them. Notably, neurons cannot replicate or regenerate once they are damaged or died [7]. Therefore, a widely investigated approach for treating neurodegenerative diseases involves either transplanting external NSCs or activating internal NSCs. Subsequently, these NSCs are induced to differentiate into neurons, facilitating the restoration of neural circuits damaged by neurological disorders [8-10].
- Astrocytes: Astrocytes are a type of glial cell that provides crucial support to neurons. Astrocytes help maintain the brain's microenvironment, regulate ion balance, and contribute to the blood-brain barrier [8, 11]. Astrocytes are involved in various processes such as neurotransmitter recycling and repair following injury [12].
- Oligodendrocytes: Oligodendrocytes play a significant role in the CNS by producing myelin, a protective sheath around axons [13]. Myelin facilitates faster electrical signal transmission, crucial for proper nervous system function [14]. NSCs are differentiated into oligodendrocytes that can contribute to post-injury remyelination, electrically insulating neuronal axons for impulse propagation, and providing trophic and metabolic support for neurons [15].
- Microglia: Microglia are the immune cells of the CNS. Microglia monitor the brain for damage, infection, and foreign substances. When needed, microglia can become activated to protect the brain by removing damaged cells and pathogens [16].

The evaluation of potential inducers on NSC differentiation is a time-consuming process, typically taking several days. This assessment is susceptible to

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various influencing factors, including molecular marking techniques, less advanced laboratory technology, and the proficiency of experimental personnel. Present methodologies may not adequately identify the factors influencing NSC differentiation, particularly regarding mechanisms that are not fully understood [17]. Additionally, current techniques rely on specific markers for each cell type, such as NeuN for neurons, GFAP for astrocytes, and Olig2 for oligodendrocytes, which are applicable only to cells at specific stages of differentiation [1, 18, 19]. As a result, early detection of NSC differentiation presents a significant challenge, hindering the progress of related technical advancements. There is an immediate need for a more efficient, precise, user-friendly, and resource-efficient method, one that minimizes subjectivity and expands our comprehension of neural development and differentiation.

In recent times, artificial intelligent (AI) has witnessed significant advancements and has exerted a profound impact on various domains. Machine learning (ML), a subset of AI, constitutes an algorithm designed for recognizing patterns and categorizing vast datasets. Deep learning (DL), a multilayered neural network that closely mimics the neural circuitry of the human brain, is employed for acquiring insights from data. The application of deep learning has been extended across diverse fields such as autonomous driving, image recognition, drug discovery, and bioinformatics [20-22]. Furthermore, the proliferation of high-throughput technology has resulted in a substantial increase in biomedical data in recent decades, encompassing genetic sequences, protein structures, and medical imaging [23, 24].

Advancements in stem cell research are increasingly being accelerated by the utilization of DL models. Integrating imaging techniques with deep neural networks (DNNs) has facilitated improved measurement and comprehension of morphological changes occurring during differentiation. These advancements aid in predicting the potential differentiation pathways of cells, annotating cells in an unbiased manner, and unraveling the identity of stem cells. DL methodologies have further been devised to reconstruct developmental trajectories from single-cell data, enhancing our understanding of stem cell fate determination at an unprecedented resolution. Additionally, these models have uncovered novel cell states that emerge during reprogramming processes. DL techniques are also expanding our ability to manipulate the behavior of stem cells, enabling control over their pattern formation and the identification of optimal culture conditions [25].

Studies have utilized DL techniques to identify various characteristics of cells, such as cell types, states, and dynamic progression, using either flow cytometry or microscopy images [26, 27]. Recently, there have been notable discoveries regarding the application of DL in observing and predicting physiological processes in stem cells. One investigation revealed that the morphology of haematopoietic stem cells changes during differentiation. DL can detect these alterations in microscopy data, enabling the early isolation of cells before the known developmental progression begins, thus predicting the development of haematopoietic stem cells in advance [28]. Another study demonstrated that machine learning can differentiate pluripotent stem cells from cells in the early stages of differentiation [29]. These findings underscore the potential extension of deep learning applications in the field of stem cell therapy. Several studies [31], [32] employed ResNet and VGG architectures. However, despite their strength and popularity, ResNet and VGG exhibit generality and high computational costs. Therefore, in this study, we propose an alternative CNN architecture.

In our work, we propose a convolutional neural network (CNN)-based method for predicting the differentiation of NSCs into Astrocytes, Neurons, and Oligodendrocytes using single-cell images. The proposed network architecture consists of four blocks, each comprising distinct layers to extract and process features at various levels of abstraction. This method aims to enable accurate and efficient classification of NSC differentiations based on single-cell image data. The main contribution of our study is to apply a CNN-based method specifically designed to predict NSC differentiation. While previous studies have employed machine learning models and convolutional neural networks for image classification tasks, the specific application to predicting the differentiation of NSCs into distinct cell types, as pursued in our work, remains largely unexplored.

The remaining portion of the paper is organized as follows. Section II introduces and describes the proposed method. Section III demonstrates and analyzes results. Finally, Section IV summarizes the research.

## II. METHOD

### *A. Data*

The single-cell image dataset utilized in this study is sourced from research [30]. The dataset consists of the following cell types: NSCs treated with astrocyte differentiation medium, NSCs treated with oligodendrocyte differentiation medium, and NSCs treated with neuron differentiation medium with retinoic acid (RA) and sonic hedgehog (SHH).

For the astrocyte dataset, the data is collected at three different time points during cell culture, specifically at 0.5 day, 1 day, and 3 days. The oligodendrocyte dataset is obtained at the following time points: 1 day, 2 days, and 3 days. Meanwhile, the neuron dataset is cultivated at 1 day, 2 days, and 5 days. The dataset consists of single-cell images in brightfield. The number of images of NSCs differentiated into Astrocyte, Neruron and Oligodendrocyte is shown in tables 1, 2, and 3, respectively.

The single-cell images were preprocessed before being fed into the convolutional neural network. We resized each image to 45 × 30 using the OpenCV package, then normalized each pixel value to be within the range

*Table 1: The number of brightfield single-cell images of NSCs differentiated into Astrocyte.*

| NSc differentiation \ Duration | 12 hours | 1 day | 2 days |
|---|---|---|---|
| Astrocyte differentiation medium | 10,269 | 23,359 | 21,838 |

*Table 2: The number of brightfield single-cell images of NSCs differentiated into Neuron.*

| NSc differentiation \ Duration | 1 day | 3 days | 5 days |
|---|---|---|---|
| Neuron differentiation medium | 11,024 | 17,912 | 8,835 |

*Table 3: The number of brightfield single-cell images of NSCs differentiated into Oligodendrocyte.*

| NSc differentiation \ Duration | 1 day | 2 days | 3 days |
|---|---|---|---|
| Oligodendrocyte differentiation medium | 5,508 | 9,478 | 12,701 |

of [0, 1]. A total of 120,924 single-cell images were split into 80% for use as training data to construct deep learning-based brightfield models, and the remaining 20% were used for model testing.

*B. Convolutional neural network*

We utilized the Xception module of the CNN architecture illustrated in Fig. 1 to perform the classification task for predictive NSCs differentiation, including astrocytes, neurons, and oligodendrocytes. The CNN architecture includes: Input layer, Convolutional layers, Batch normalization, ReLU activation, Separable convolutions, Max pooling, Average pooling and Fully connected:

*(1) Convolutional layers:* These layers are responsible for extracting various features and patterns from the input images, which utilize filters to perform convolution operations, capturing important spatial hierarchies within the data.

*(2) Batch normalization:* Integrated after each convolutional layer, batch normalization standardizes the outputs of the previous layer. This helps stabilize the training process and accelerates convergence, ensuring efficient and stable learning.

*(3) ReLU activation:* Rectified Linear Unit (ReLU) activation function introduces non-linearity into the network, allowing it to learn complex relationships and representations within the data. It helps the network model complex phenomena, leading to improved predictive performance.

*(4) Separable convolutions*: These convolutions are utilized to efficiently capture spatial information within the data while reducing computational complexity. By separating the process into depthwise and pointwise convolutions, it enables the network to learn complex spatial patterns more effectively.

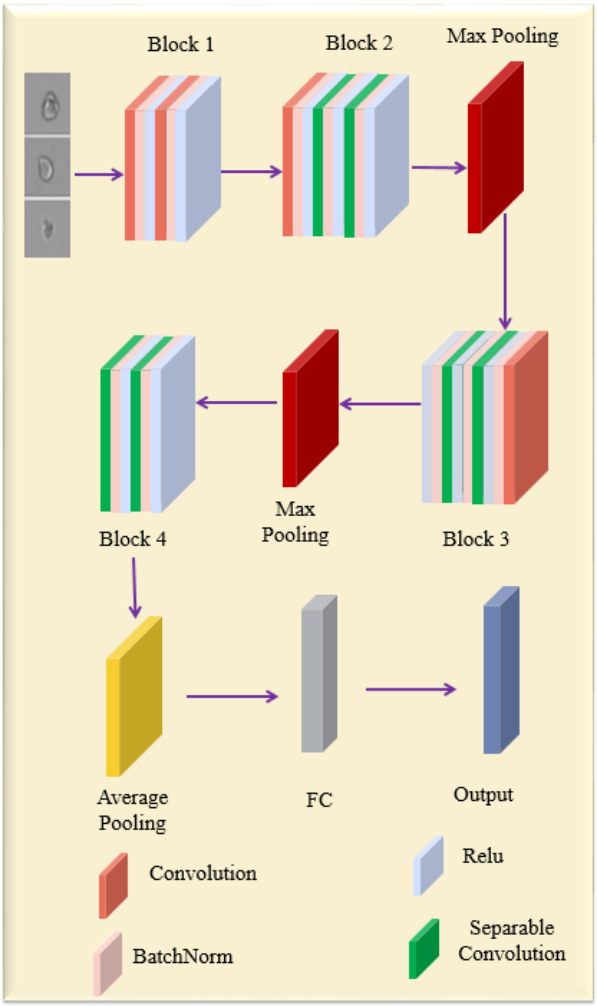


*Fig. 1: The CNN architecture of the proposed method*

*(5) Max pooling and Average pooling*: Max pooling layers downsample the feature maps, retaining the most significant features, while average pooling layers compute the average of the values within a certain kernel size. Both pooling operations help in reducing the spatial dimensions and controlling overfitting

*(6) Output layer* includes a fully connected layer followed by a softmax activation function, providing a probability distribution over different cell types, thus enabling the model to predict the differentiation status of NSCs into astrocytes, neurons, or oligodendrocytes.

*C. Performance evaluation*

To evaluate performance of proposed model, we use Accuracy (Acc), precision (Pre), specificity (Sp) and Recall. The first one, Accuracy, refers to the ratio of correctly predicted observations to the total observations, providing an overall assessment of the model's correctness in predicting all cell types. Precision, on the other hand, measures the fraction of relevant instances among the retrieved instances, allowing us to understand how many

of the predicted instances are relevant to the specific cell types. Specificity indicates the proportion of actual negative cases that are correctly identified as such, assisting in gauging the model's effectiveness in correctly identifying true negatives. Recall measures the fraction of true positive predictions out of all actual positive instances, serving as an indicator of the model's capability to detect all relevant cases of Astrocyte, Neuron, and Oligodendrocyte without missing any. The formula of this parameter is calculated as follows:

$$Acc = \frac{TP + TN}{TP + TN + FP + FN} \quad (1)$$

$$Pre = \frac{TP}{TP + FP} \quad (2)$$

$$Sp = \frac{TN}{FP + TN} \quad (3)$$

$$Recall = \frac{TP}{TP + FN} \quad (4)$$

These parameters are normally defined for binary classification problems where the outcome is either "positive" or "negative". As, we have three classes and dealing with the multi-class problem, so we computed, Acc, Pre, Sp and Recall, while calculating TN (True Negative), TP (True Positive), FP (False Positive), and FN (False Negative) of each class separately. Table 4 and 5, shows various performance measures were obtained from the confusion matrix.

*Table 4: Confusion matrix*

| Confusion Matrix | | Predicted | | | False Negative (FN) |
|---|---|---|---|---|---|
| | | Class 1 | Class 2 | Class 3 | |
| Actual | Class 1 | A | B | C | B+C |
| | Class 2 | D | E | F | D+F |
| | Class 3 | G | H | I | G+H |
| False Positive (FP) | | D+G | B+H | C+F | |

*Table 5: Computing different performance measures from confusion matrix*

| | Class 1 | Class 2 | Class 3 |
|---|---|---|---|
| Pre | A/(A+D+G) | E/(B+E+H) | I/(C+F+I) |
| Sp | (E+I)/ (D+G+E+I) | (A+I)/ (B+H+A+I) | (A+E)/ (C+F+A+E) |
| Recall | A/(A+B+C) | E/(D+E+F) | I/(G+H+I) |

## III. SIMULATION RESULTS AND DISCUSSION

The convolutional neural network (CNN) model was trained and tested on a dataset of single-cell images, with 96,740 images used for training and 21,184 images used for testing. Each image was resized to 45x30 pixels and underwent appropriate normalization and preprocessing before being fed into the model. The model was designed to classify the cells into three distinct types: Astrocytes, Neurons, and Oligodendrocytes. The testing results were documented in the confusion matrix presented in Table 6.

Table 6 illustrates the confusion matrix derived from the CNN model's performance on the testing set, with Class 1 representing Astrocyte, Class 2 representing Neuron, and Class 3 representing Oligodendrocytes. The confusion matrix provides a detailed breakdown of the model's predictive capabilities for each cell type. It outlines the number of instances correctly and incorrectly classified within each class, enabling a comprehensive evaluation of the CNN model's performance. The model demonstrates robust performance, particularly in predicting Class 2 (Neuron) and Class 3 (Oligodendrocyte), as evidenced by the high numbers of correct predictions.

*Table 6: Confusion matrix of CNN model on testing set (Class 1: Astrocyte, Class 2: Neuron, Class 3: Oligodendrocyte)*

| Confusion Matrix | | Predicted | | |
|---|---|---|---|---|
| | | Class 1 | Class 2 | Class 3 |
| Actual | Class 1 | 10227 | 651 | 215 |
| | Class 2 | 304 | 6750 | 500 |
| | Class 3 | 898 | 0 | 4639 |

Further insights into the performance of the model are revealed in Table 7, which presents additional performance metrics. The accuracy rates for predicting Astrocyte, Neuron, and Oligodendrocyte are 91.27%, 93.69%, and 93.06%, respectively. Moreover, the precision, specificity, and recall percentages provide a deeper understanding of the model's predictive capabilities for each cell type, indicating a strong ability to discriminate between different cell types.

*Table 7: The performance of predicting NSCs differentiation*

| | Astrocyte | Neuron | Oligodendrocyte |
|---|---|---|---|
| Acc (%) | 91.27 | 93.69 | 93.06 |
| Pre (%) | 89.48 | 91.20 | 86.65 |
| Sp (%) | 90.45 | 95.8 | 95.96 |
| Recall (%) | 92.19 | 89.36 | 83.78 |

Besides, to evaluate the differentiation prediction of NSCs, we assessed the time-dependent prediction of collected data cells. The results are illustrated in Fig. 2, indicating that after 1 day of cultivation, the model detected the differentiation into Astrocyte, Neuron and

Oligodendrocyte cells with high accuracy of 93.86%, 90.89% and 80.6%, respectively.

Transplanting NSCs offers promising options for CNS recovery, but guiding their differentiation into specific cell types is tough. Biomarkers are commonly used to track the changes, but the exact process of neurogenesis, especially the early stages of neuron formation, remains unclear. This makes it challenging to identify the direction of differentiation early on. A reliable identification process is necessary to develop effective treatments for neurodegenerative diseases and neurological injuries, regardless of the treatment pathways. Though advanced tools aid data collection, understanding the data is difficult due to current device limitations. Existing methods rely heavily on human understanding, making it tough to identify small changes in cell shape or predict drug interactions [17]. These results of our proposed model illustrate the efficacy of the CNN model in accurately predicting the differentiation of NSCs into the specified cell types. The high accuracy rates and robust performance metrics demonstrate the model's potential for precise identification and classification of cell types, thereby presenting promising prospects for the advancement of cell-based treatments and therapies for various central nervous system disorders.

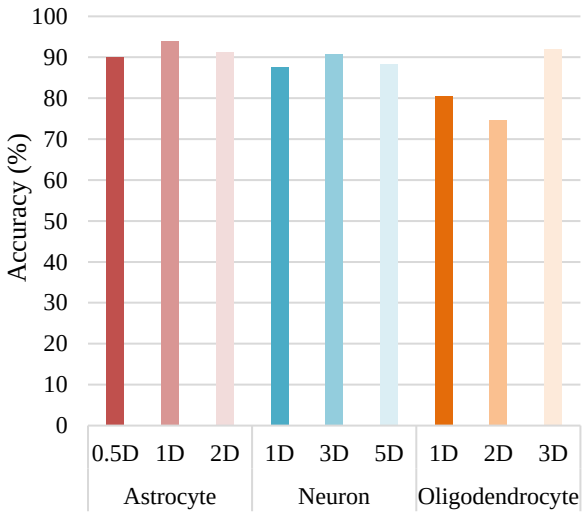


*Fig. 2: Accuracy of each testing set brightfield model*

*Table 8: Comparison of the accuracy performance of the proposed CNN architecture with other method for predicting 1 day of nscs differential cultivation.)*

| Ref | Astrocyte | Neuron | Oligodendrocyte |
|---|---|---|---|
| [17] | 95.86% | 82.73% | 80.59% |
| Our | 93.86% | 90.89% | 80.6% |

To evaluate the performance of our model in comparison with other research, we compare the accuracy performance of the proposed CNN architecture to that of the reference method for predicting the 1-day differential cultivation of NSCs, as presented in Table 8. Regarding specific cell types, the proposed CNN architecture achieves an accuracy of 93.86% for Astrocyte prediction, slightly below the reference method's 95.86%. However, it outperforms the reference with 90.89% accuracy for Neuron prediction compared to 82.73%. Both methods exhibit similar accuracy for Oligodendrocyte prediction, with the proposed CNN architecture at 80.6% and the reference method at 80.59%. Besides, our CNN architecture is simpler than the one presented in research [17]. These results suggest the competitiveness of the proposed CNN architecture in predicting NSCs differentiation, particularly excelling in Neuron prediction, while maintaining comparable accuracy in other cell types.

## IV. CONCLUSION

In summary, this paper has introduced a method utilizing CNN techniques to accurately predict the differentiation of Neural Stem Cells into Astrocytes, Neurons, and Oligodendrocytes, employing single-cell brightfield images. With its capacity to predict NSC differentiation within a day, the model presents a promising avenue for investigating the effects of various substances on NSCs. The results demonstrate the efficacy and reliability of the proposed approach, paving the way for improved understanding and monitoring of NSC differentiation dynamics. This technique holds promising implications for the advancement of cell-based therapies for various central nervous system disorders.

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## DỰ ĐOÁN SỰ BIỆT HÓA TẾ BÀO GỐC THẦN KINH SỬ DỤNG HÌNH ẢNH TỪNG TẾ BÀO DỰA TRÊN HỌC SÂU

***Tóm tắt:*** Quá trình phân biệt tế bào gốc thần kinh (NSC) thành tế bào thần kinh là quan trọng cho việc phát triển các phương pháp điều trị tập trung vào tế bào tiềm năng cho các rối loạn hệ thống thần kinh trung ương. Tuy nhiên, việc dự đoán, nhận diện và tiên đoán sự phân biệt này là phức tạp. Trong nghiên cứu này, chúng tôi đề xuất việc triển khai mô hình mạng nơ-ron tích chập để nhận diện có thể dự đoán vận mệnh của NSC, sử dụng hình ảnh đơn tế bào sáng trường. Kết quả chứng minh hiệu quả của mô hình trong dự đoán sự phân biệt NSC thành astrocytes, tế bào thần kinh và oligodendrocytes, đạt tỷ lệ chính xác lần lượt là 91.27%, 93.69% và 93.06%. Hơn nữa, mô hình đề xuất của chúng tôi hiệu quả trong việc phân biệt giữa các loại tế bào khác nhau ngay cả trong ngày đầu tiên của quá trình nuôi cấy.

***Từ khóa***: Biệt tế bào gốc thần kinh, Mạng nơ-ron tích chập, Hình ảnh đơn tế bào, Tế bào gốc, Học sâu.

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