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Deep Learning: Automating the Future of Stem Cell Bioimage Analysis

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ABSTRACT

The use of deep learning has been explored in various fields of science. Deep learning utilizes artificial neural networks which contain neurons arranged in layers to analyze data and generate predictions. Several deep learning architectures have been used in image recognition and analysis, including bioimage analysis in stem cell research. Stem cells, with their differentiation potential, are widely used in drug testing, disease modeling, and regenerative treatments. In stem cell research, it is essential to identify and track which cell lineage stem cells have differentiated into. Until recently, this has been done with the use of molecular labeling and manual methods, which are mostly subjective and error-prone. The use of deep learning to identify and classify stem cells offers potential solutions of automation, and cost-effectiveness, in addition to high performance accuracy. This article summarizes how deep learning can be used in identifying stem cells, along with their current limitations.

Keywords: deep learning; stem cell; bioimage analysis.

INTRODUCTION

Recently, there has been a significant surge of research involving machine learning (ML) technologies in various science disciplines. ML has enabled scientists to push beyond the boundaries of what they could even think was possible decades ago. It has penetrated almost all scientific fields, from speech recognition to screening for new anti-cancer drugs.[1–3] Numerous factors might have contributed to this massive explosion of ML involvement in science, such as how high, intensive computing power has become more accessible due to the rapid advancement of various computer graphic processor units (GPUs) and central processing units (CPUs).[4] Indeed, high computing power is required because ML utilizes the ability of artificial neural networks (ANNs), which comprise many layers of basic computing cells or "neurons" to analyze results from given data input.[3,4] The primary goal of ML is to generate correct predictions of new, unknown data based on the provided input data. In order to accomplish this, we create mathematical models or algorithms which can be trained and optimized by feeding them with training data or "experiences". A branch of ML study called deep learning (DL) has gained much attention in recent years. Deep learning uses ANNs with multiple layers, whose performance can self-optimise as we increase its training data due to end-to-end training.[1,4]

One of the rapidly evolving life science fields affected by DL technologies is bioimage analysis for stem cells. The stem cell is a class of undifferentiated progenitor cells, which possess the potential to differentiate into other particular cells while also showing the capacity to self-renew.[5] Based on their origins, they are classified into embryonic stem cells (ESCs), which are pluripotent stem cells, adult stem cells (ASCs), which are multipotent and lastly, induced pluripotent stem cells (iPSCs), which are derived from reprogrammed somatic cells.[5,6] In studying stem cells, it is essential to perform cell differentiation lineage tracing. This is usually done by molecularly labeling the cells. DL offers potential solutions to speed up stem cell study by providing means of automated cell identification and classification from microscopy images, without the use of molecular labeling.[5,7] This essay will attempt to summarise the basics of how we can utilize DL in stem cell research, along with their applications, current limitations, and what they might bring to the future of this developing field of science.

BASIC PRINCIPLES

Basic Principles of Deep Learning

DL involves the use of a deep neural network. A deep neural network comprises building units or neurons arranged in multiple hidden layers. Neurons within the same layer are disconnected from each other, while inter-layer neurons are connected adjacently through internal links or activation functions (Figure 1).[8] Input datasets of a deep neural network are commonly divided into the training phase data (training set) and the test phase data (test set). Every neuron of the hidden layers in Figure 1 processes its input, then models a decision by using bias (b) and weight vector (W) in a connection function:

$$h_{W,b}(X) = f(W^T X + b)$$

Based on the above formula, the input neurons carry out data processing by multiplying their particular weight vector matrix and then adding a bias. The function output of this process is then relayed to the next neurons in the adjacent layers as input function. This relationship, in which the input features are extracted to the adjacent layer, enables further refinement and optimization of the input features.[2,8]

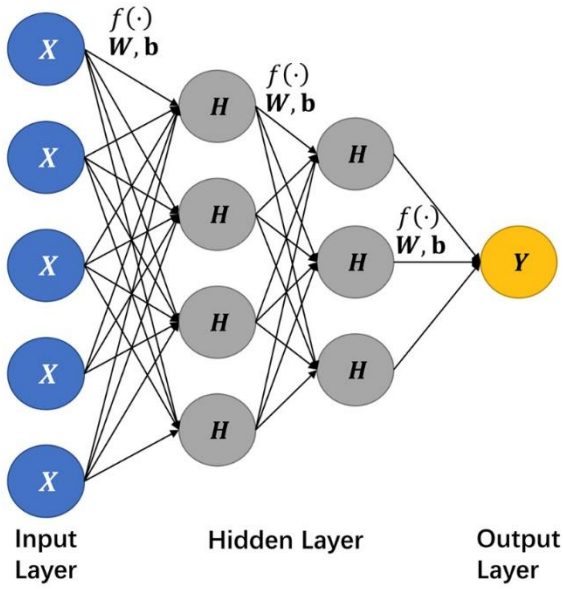

FIGURE 1: Structure of a typical deep learning network. f(.) is the activation function. X represents the input layer, H and Y represent the hidden layers and output layer, respectively.[8]

As mentioned before, the input dataset is separated into training and testing sets. The overall analysis procedure of the network relies on its training, where the network adjusts its own weights or other parameters based on several learning paradigms (activation/rectification functions) to generate the appropriate predictions.[8] To assess the training process, testing, validation, or performance comparison can be carried out. Testing and/or validation are used for error estimation and further improvements (Figure 2). The error here is expressed as the disparity between the predicted values and the correct true values, which can be computed using a loss function. In turn, loss functions are then influenced and adjusted by backpropagation of the testing results.[2,8] In general, the whole training process revolves around the use of activation functions to search through the layers until the ending threshold, and loss function minimisations to find the ideal parameter combinations. Specifically, activation functions here dictate the status of the neuron output (active/inactive) in relation to neurons in the next layer.[8] To summarise, DL uses hierarchical layers of ANNs to extract high-level features from low-level input data. Consequently, DL is less dependent on the user experience, as it uses end-to-end training of direct input-output recognitions between ANN layers to generate feature representations or predictions.[4,5]

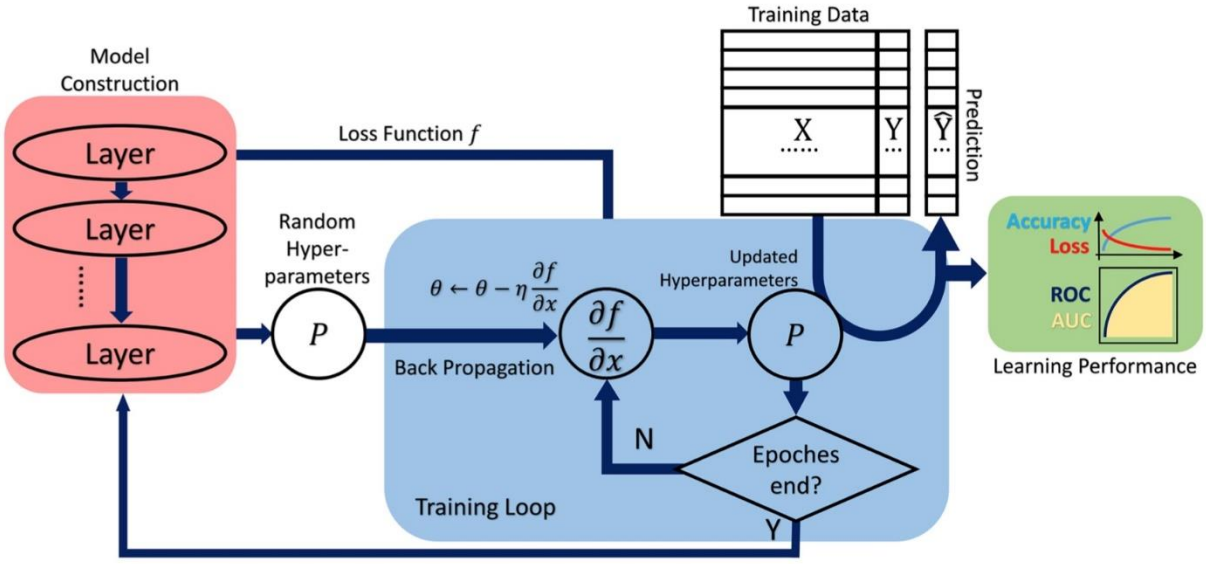

FIGURE 2: The analysis process of a common deep learning network, which includes training datasets, model construction, training loop for parameter optimization, and performance assessment/validation. (Tang et al., 2019).

Convolutional Neural Network

There are several common deep neural network architectural types, including convolutional neural network (CNN), recurrent neural network (RNN), deep belief network (DBN), and autoencoder (Tang et al., 2019; Maier et al., 2019). For stem cell research, CNN is one of the most prevalently utilized deep neural network models.[5,7]

CNN is suitable for processing multiple arrays of tasks, such as object recognition, image aggregation, and image classification. Thus, it is frequently used in image-processing tasks.[8] The architecture of CNN reportedly dates back to 1980, when a pattern recognition neural network model called "Neocoginitron" was developed.[5]

In general, CNN workflow is split into two categories: feature extraction and classification. Feature extraction task is carried out by convolution layers, nonlinear activators, and pooling layers, whereas deep neural network classifiers perform the classification task.[5,8] Convolution layers extract various sizes of feature maps based on the input data by applying some filters.[8] These layers start with recognizing lines, edges, and blobs of an image, which are the simpler features of the image. Then, the deeper neurons in the subsequent layers will extract and identify more complicated features. The adjacent pooling layers will then perform reduction or subsampling to this convolution layer output and create pixel column vectors that extend across the layers' depth.[2,5,8] High-level feature extractions are possible by repeating these convolution and subsampling processes through the use of activation functions, which turn neurons "on" or "off" in relation to the adjacent layer.[8]

The stretched pixel column vectors from the pooling layer are then relayed to the fully connected layers, where final decisions for object classification are made.[5,8] Therefore, as we increase the number of layers, we can also increase the feature levels that the network will extract.[8] Lastly, a loss layer will measure the errors from the predicted values using a loss function. This loss layer can self-adjust its weight for the whole network. CNN performance can be enhanced by training, in which backpropagation of the error values to the previous layers improves the feature extraction process.[5,8] Because of its feature extraction, classification, and self-learning ability, the use of CNN has been explored extensively in biomedical science, from analyzing CT scans and MRI images of various diseases to predicting cell gene expression.[5,7,8]

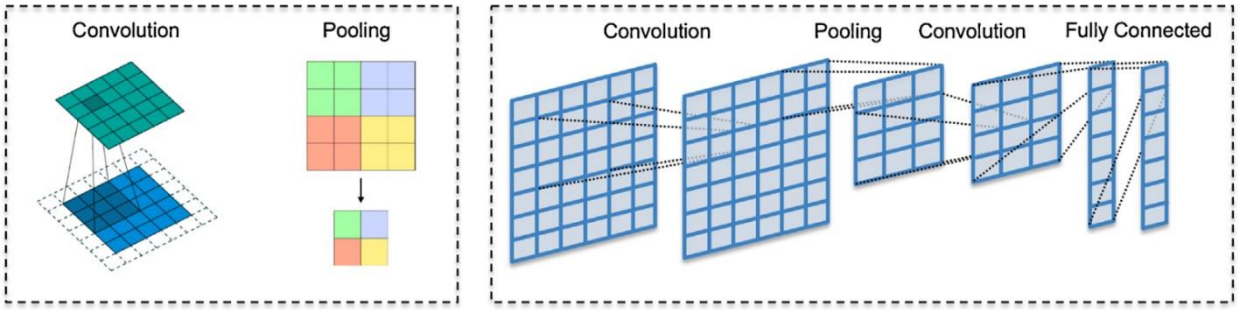

FIGURE 3: Structures of a CNN. The convolution layer recognizes objects, whereas the pooling layer subsamples the output of the convolution layer. Convolution and pooling layers are usually placed alternatively before the fully connected layer.[2]

APPLICATIONS OF DEEP LEARNING

CNN in Identification of Endothelial Cells Differentiated from Induced Pluripotent Stem Cells

Since its introduction in 2006, induced pluripotent stem cells (iPSCs) have been prevalently used in drug testing, disease modeling, and regenerative medicine.[7,9] A number of advantages of using iPSCs might have contributed to this, such as their differentiation potential and the ability to acquire iPSCs without any ethical concerns, as they are derived from reprogrammed somatic cells.[5,7] Observing and analyzing morphological changes is essential in studying iPSCs, as they go through various phases of differentiation. Thus far, this analysis depends on human expertise, which is time-consuming, subjective, and error-prone. To overcome this limitation, Kusumoto et al. have explored the role of CNN in identifying and classifying endothelial cells differentiated from iPSCs.[10]

First, the group differentiated the iPSCs into endothelial cells, and then fluorescent staining was performed to verify the differentiation, in which the differentiated cells would be stained. Phase contrast and binarised immunofluorescent images of these cells were then obtained as the datasets.

The group managed to acquire a total of 800 images, of which 640 were included in the training phase, and 160 were used for validation. Afterward, phase contrast images were extracted for random input blocks. Furthermore, to create target blocks that match with the input blocks, matching binarised immunofluorescent and phase contrast images were also extracted. These entire target blocks were then binarised based on the white-black pixels ratio to give predictive values of "stained" (1) or "unstained" (0). Finally, a small CNN (LeNet) and a large CNN (AlexNet) were used to analyze the input blocks. The goal was to create predictions from the phase contrast images and then compare them with the binarised target blocks. The small network was used to adjust the input and target block size, number of blocks, as well as staining threshold value in order to optimize its prediction. Subsequently, to train the whole network, the large network was used as a comparison to the small network results. The differences in these comparisons were then calculated and backpropagated for re-binarisation of the target blocks to improve the whole network performance. Eventually, K-fold cross-validation was used to validate this optimization process. Figure 4 summarises the approach used in this study.

The overall performance of the network was evaluated using parameters, such as accuracy (correct prediction fractions) and F1 score (recall and precision amounts). Satisfactory performance of >90% accuracy and >75% F1 score was achieved by using this CNN workflow approach. However, there were some instances where this network performed poorly.

For example, in the case of autofluorescences found in areas with dense colonies, non-specific fluorescences, and faint staining. Nevertheless, this study indicates that automated identification and classification of iPSC-differentiated endothelial cells based solely on morphological features is possible through the use of CNN.

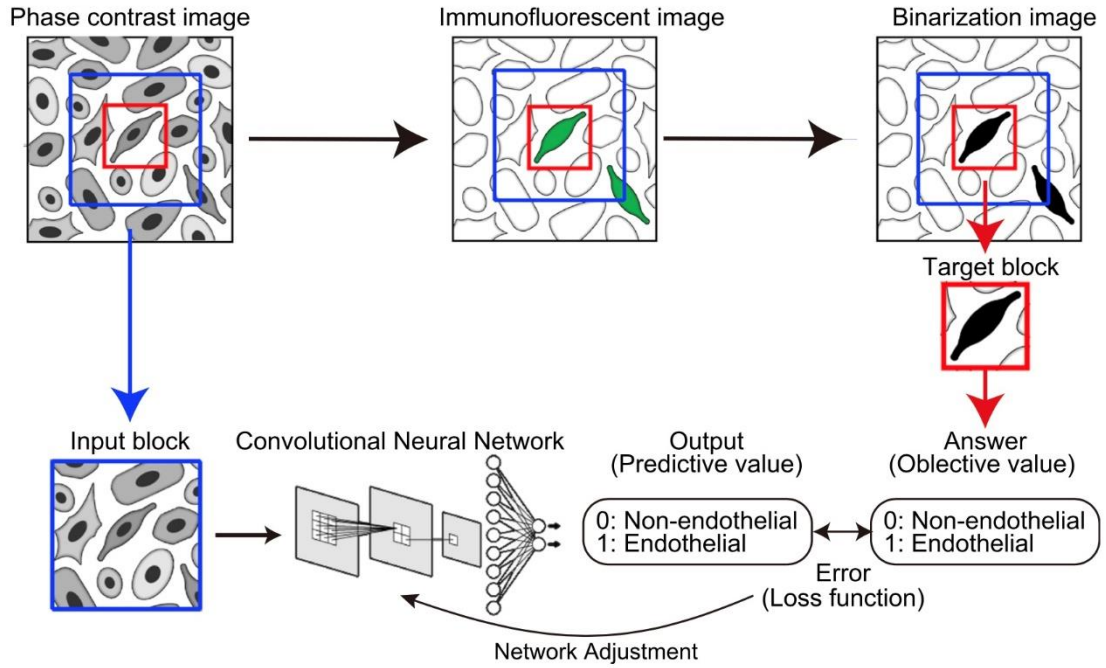

FIGURE 4: Utilisation strategy of CNN for identification and classification of iPSCs-derived endothelial cells. Input blocks are obtained from phase contrast images, then analyzed by CNN to create "stained" (endothelial cells) or "unstained" (non-endothelial cells) prediction. Matching features of the immunofluorescent images and phase contrast images (not illustrated) are then extracted and binarised to create target blocks as the correct answers. Analyzed input block predictions are contrasted to the true answers. The differences in the predictions are then used by the network to self-adjust its weight, improving the overall performance.[10]

CNN in Early Identification of Differentiated Mice Pluripotent Stem Cells

A more compelling performance of CNNs in detecting differentiated stem cells was demonstrated by Waisman et al. The group sought to train CNN models to identify and distinguish epiblast-like cells (EpiCLs) from undifferentiated mice ESCs (mESCs).[11] These EpiCLs were acquired by inducing mESCs differentiation. CNN training was performed at various time points from the onset of differentiation, and by using this approach, the networks were able to produce an impressive accuracy of >99% during the 24-48 hours period. Moreover, the start of the correct predictions could be traced back to just 20 minutes after the differentiation was induced. First, light microscope images of differentiating EpiCLs and undifferentiated mESCs were taken (Figure 5). These images were taken at 0, 2, 6, 12, and 24 h time points. The undifferentiated mESCs were cultured in a special medium called "2i+LIF" to maintain their undifferentiated condition. Next, the group used and trained ResNet50 CNN architecture, where around 800 images for both cell groups were used for the training phase. Additionally, 200 images and 50 images per group were used for testing and validation, respectively. After a satisfactory performance was achieved using this approach, the group proceeded to add more images to the datasets with increased cell density and used another CNN architecture (DenseNet) to enhance the network performance. Both networks were able to show high accuracy without the need to perform image preprocessing or to increase the number of layers in each network.

To analyze the prediction mechanisms behind the ResNet50 and DenseNet networks, the group translated activation layers in each network's hidden layers into pixels. A number of 49 layers with activations that correspond to the translated pixels were found among the 168 layers in ResNet50. Initially, the original images that were fed to this network were 480 rows by 640 columns in size and contained 3 channels. In the final activation layer, which would feed the fully connected layers for predictions and optimizations, these images were found to be smaller in size (8 rows by 3 columns), but the depth (channels) of these images had increased to 2,048, as the network stretched the pixel column vector. In contrast, images of the final activation layer in DenseNet were found to be larger in size and lower in depth, as the pixel column vector in DenseNet was stretched and then condensed by the network. This analysis method successfully showed the different

mechanism of each CNN architecture in performing the convolution, identification, classification, and prediction task.

Finally, the group used data from 3 additional experiments to validate the predictions generated by both networks, which also resulted in a high accuracy value of 0.998 for DenseNet and 0.996 for ResNet50. Furthermore, the accuracy at a time point as early as 20 minutes after the differentiation induction was also found to reach above 80%. This was confirmed by using images in the 1-h dataset and calculating the accuracy in 10-minute intervals. However, there are several challenges and limitations in using this strategy for stem cell identification.

First, lower probabilities of detecting the 2i+LIF group (undifferentiated cells) were found in both networks, meaning that both networks were biased more toward generating predictions for differentiated cells. The group suggested this was due to the fact that these networks used object borders to interpret an image. Therefore, it may be easier to identify differentiated cells, as they showed more apparent morphological features, such as spindles and plasma membrane protrusions. Lastly, high computing power might be needed since the CNNs used in this study contain a substantial number of layers, and a minimum number of 1400 images are required to generate accurate predictions. Nevertheless, this study shows how CNNs can be utilized to generate highly accurate and early predictions for stem cell identification.

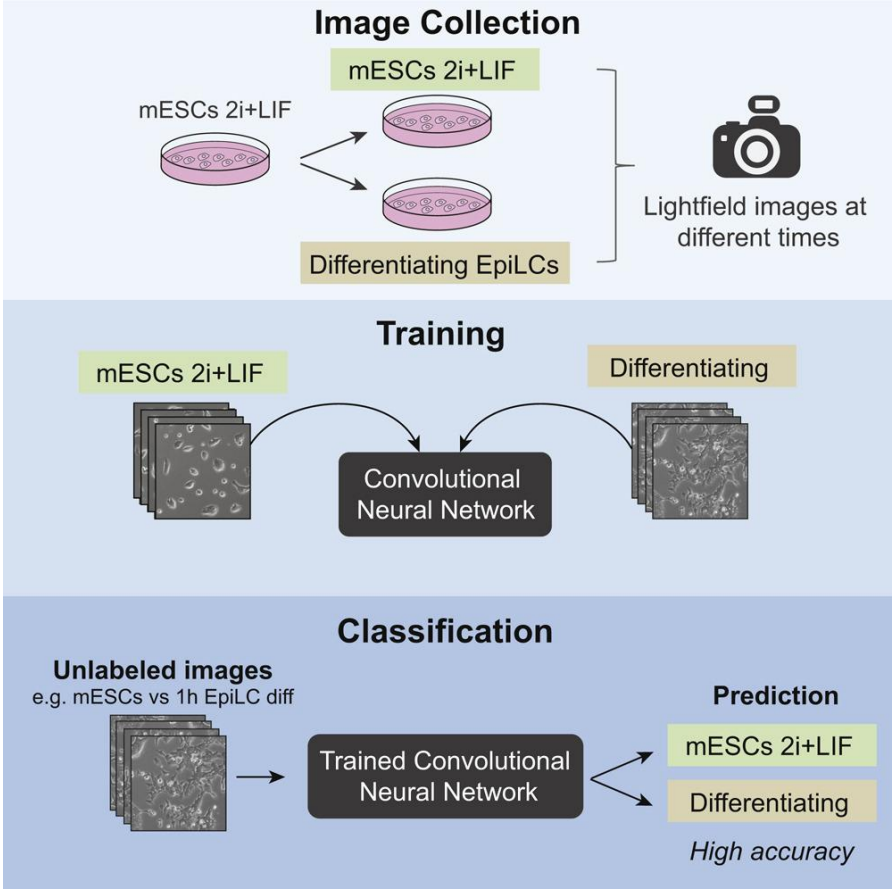

FIGURE 5: An overview of the experiment method used in this study. Two types of CNN architecture were used for analysis: ResNet50 and DenseNet.[11]

FUTURE DIRECTIONS

The two studies mentioned previously demonstrate how DL has been successfully used for bioimage analysis in stem cell research. With an average accuracy of >90%, these deep-learning models can easily exceed human expertise in identifying stem cells. Advantages, such as automation, rapid and accurate detection, and low cost compared to molecular procedures, will encourage the prevalent use of deep learning. However, sensitivity/specificity issues in identification tasks, high computing power, and storage needed for analyzing a large size of training datasets still become challenges that prompt further improvements.[10,11]

More training, for example, by using specific datasets which deliberately train the DL network for false positives or negatives may be used to increase its accuracy.[10] In addition, open-set recognition networks, which also consider and adapt to new, "never-trained-before" data in real-time, should also be explored.[3] Cloud computing services dealing specifically with bioimaging data analysis and storage can be developed to overcome the current computing hardware limitations faced by many laboratories. Nevertheless, as the technologies in computer hardware and ML science advance, the goal of creating DL systems that are cost-effective,

user-friendly, with efficient and optimal performance will become more feasible.[3] With its ability, the prevalent use of DL in the future will bring significant impacts to biomedical science in general.

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