

Classification of Global Microglia Proliferation Based on Deep Learning with Local Images

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ABSTRACT

Microglial cell proliferation in neural tissue (neuroinflammation) occurs during infections, neurological disease, neurotoxicity, and other conditions. In basic science and clinical studies, quantification of microglial proliferation requires extensive manual counting (cell clicking) by trained experts (~ 2 hours per case). Previous efforts to automate this process have focused on stereology-based estimation of global cell number using deep learning (DL)-based segmentation of immunostained microglial cells at high magnification. To further improve on throughput efficiency, we propose a novel approach using snapshot ensembles of convolutional neural networks (CNN) with training using local images, i.e., low (20x) magnification, to predict high or low microglial proliferation at the global level. An expert uses stereology to quantify the global microglia cell number at high magnification, applies a label of high or low proliferation at the animal (mouse) level, then assigns this global label to each 20x image as ground truth for training a CNN to predict global proliferation. To test accuracy, cross validation with six mouse brains from each class for training and one each for testing was done. The ensemble predictions were averaged, and the test brain was assigned a label based on the predicted class of the majority of images from that brain. The ensemble accurately classified proliferation in 11 of 14 brains ($\sim 80\%$) in less than a minute per case, without cell-level segmentation or manual stereology at high magnification. This approach shows, for the first time, that training a DL model with local images can efficiently predict microglial cell proliferation at the global level. The dataset used in this work is publicly available at: tinyurl.com/20xData-USF-SRC.

Keywords: Convolutional Neural Network, Classification, Microscopy

1. INTRODUCTION

A wide range of basic research and drug discovery studies require quantification of microglial cell proliferation on stained tissue sections to better understand and treat neurological conditions. Traditionally, quantitative studies of microglial proliferation, i.e., increased microglia cells in an anatomically defined region of interest, are done by a well-trained data collector using computer-assisted stereology methods.¹ However, a simple quantification of microglia cell proliferation in a single mouse brain region requires about two hours spent analyzing stained tissue sections. Further limitations are that the data generated by these studies are prone to low inter-rater agreement due to human factors such as subjective decision-making, variable training and experience of data collectors, fatigue, etc.² Machine Learning offers a variety of automatic approaches to enhance the accuracy, reproducibility, and efficiency of assessing microglial cell proliferation on stained tissue sections while reducing the human requirements for this work.

Our group proposed one of the first methods to automate stereology methods using a hand-crafted Adaptive Segmentation Algorithm (ASA) for segmenting cells at high magnification (60x to 100x). As described in³

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ASA uses a Gaussian Mixture Model (GMM), morphological operations, Voronoi diagrams, and watershed segmentation for automatic cell counts on extended depth of field (EDF) images created from volumes of z-axis stacks as input.⁴ Early applications of this method showed reasonable performance with an error rate of around 11%, high reproducibility and 5x increases in throughput relative to manual stereology counts. We then improved all performance metrics with the development of a deep learning (DL) approach to perform the cell-level segmentations for calculation of total cell number using the unbiased optical fractionator method.³ Alahmari et al. proposed the use of CNNs for segmentation of brain cells (NeuN-immunostained neurons) in the neocortex (NCTX) of mouse brains.² This method used ASA to automatically generate and verify masks which were then used to train a U-Net model⁵ to segment cells with post-processing according to unbiased cell counting rules to make stereology-based estimates of total number.^{2,3} This approach used an iterative deep learning technique in which a trained human expert verifies previous predictions that are then used for training the image sets of subsequent models. This work showed that within five iterations the error rate difference compared to a trained professional fell to below 1%, i.e., less than a tenth of the error by the ASA method. Though the CNN performed well for segmenting cells at high magnification, the iterative deep learning process still required extensive human-in-the-loop input to verify the ASA-generated masks. For many studies focused on assessing microglial cell proliferation, a simple automatic classification of global proliferation based on low magnification images would be an attractive alternative to automatic segmentation of cells at high magnification. This is particularly true for quantifying changes such as proliferation at a global level in response to functional changes, e.g., inflammation, malignancy, rather than fine changes at the cellular and subcellular levels.

To address this issue, we trained our model with low magnification images (20x) of Iba1-immunostained microglia cells using a snapshot ensemble of CNNs to classify the proliferation of the total number of microglia cells in the NCTX of mouse brains. The model can classify each image as belonging to low or high total numbers of microglial cells at the global level in NCTX, without the need for human-in-the-loop training, cell-level segmentation, or manual stereology at high magnification. Because the approach requires little effort or technical expertise by the data collector, it has the potential to accelerate the throughput of microglial proliferation studies in many scientific disciplines.

2. METHOD

Animal tissues for this study were the Dp16 mouse model of Down syndrome (trisomy Hsa21) with APP gene overexpression; and sex- and age-matched littermate controls (2N). Serial 40 μm -thick sections were cut through the entire NCTX and stained using standard Iba-1 immunostaining to label microglial cells. The total number of Iba-1 microglial cells in NCTX were quantified by an expert technician using the manual version of the unbiased optical fractionator⁶ in a computerized stereology system (Stereologer[®], SRC Biosciences, Tampa, FL, USA). Cases were sorted from high to low proliferation based on the total number of microglial cells and seven (7) cases were selected from each extrema, i.e., n=7 cases each with the highest and lowest total number of microglial cells in NCTX. For the low cases the total number of cells ranged from 405,200 to 452,550 and for the high cases from 638,670 to 714,740. From these cases an image dataset was automatically collected at 20x magnification in a systematic-random set of sections through each NCTX as we have detailed previously.⁷ A minimum of 380 low magnification (20x) fields of microglia cells were imaged at systematic-random locations in about 15 sections through NCTX of each brain. Each case (mouse brain) received a ground truth (GT) label of either high or low proliferation of microglia cells, i.e., high or low total number of cells in NCTX, with the same label applied to all images from that brain. Images were not individually labeled as high or low total number of cells due to the difficulty, if not the impossibility, of doing so even with a minimal level of confidence by a well-trained expert. Pre-processing included color thresholding to remove all images that contained less than 50% tissue in frame and center cropping to a size of 512x512 pixels for input to the CNN. The images were further pre-processed to convert from RGB to grayscale using a correlation-based approach.⁸ The method was assessed by cross validation with training on n=5 cases from each class, validated on n=1 from each class, and tested on n=1 from each class. Finally, to increase the quantity of training data, all training images were augmented with two elastic deformations and rotation by 90 degrees.

Our work began by trying a few off the shelf architectures like VGG16⁹ and DenseNet,¹⁰ however performance was low due to the large number of parameters in these models. Therefore a custom CNN architecture was

developed in Keras¹¹ with TensorFlow¹² backend, as shown in Figure 1. All convolution layers used ReLU activation, our two fully connected layers used ReLU with the addition of L2 regularization, the output consists of a softmax activation function, and finally the model was trained using Stochastic Gradient Descent (SGD) for optimization. To enhance performance, we used the snapshot ensemble approach proposed in¹³ with a cyclic learning rate in the form of cosine annealing to produce variability in the models as training proceeds. This technique allows for training a single time and saving intermediate models, which then can be used in an ensemble without the need for training the model multiple times. We trained 110 epochs with a cycle of 10 epochs and a maximum learning rate of 0.004. This approach produces 11 models used to predict on the images then those 11 predictions were averaged for a final classification.

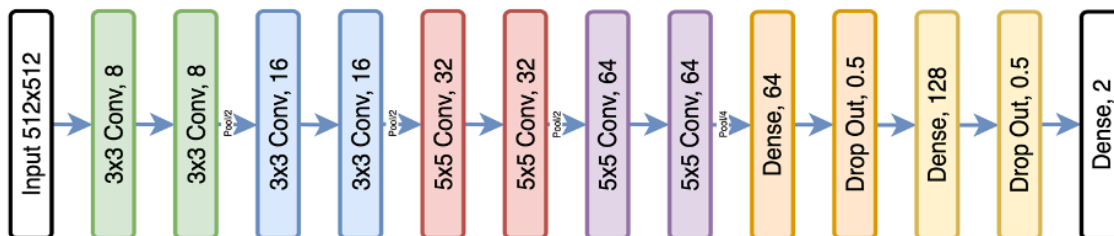


Figure 1. Network Architecture

3. RESULTS

The goal of our model is to correctly predict whether microglial cell proliferation exists at the global level, i.e., in the mouse NCTX, based on ensemble training with local images of microglia cell densities at low (20x) magnification. The correct labeling is based on the known proliferation of microglial cells in NCTX from a priori stereology studies using the ordinary optical fractionator method with manual counting at high magnification. A threshold of 50% of an animals images belonging to a single class was used to assign the class when testing. The reason for this assignment strategy is that not all low magnification fields of microglia cells are expected to match its global class label, the class assigned to each mouse required at least half of the total images of microglial cells in NCTX to be automatically classified as either high or low proliferation at the global level. The 50% threshold for assigning the global label avoids misclassifying cases where the label does not match many images within a mouse. For instance, Figure 2 shows examples of images where local densities appear to fit their training labels with high proliferation on the left and low proliferation on the right. In contrast, Figure 3 shows two images with high and low proliferation at the global level though they both appear to be low density at the local level. Similarly, in Figure 4 two images are shown with high and low proliferation at the global level though they both appear to be high density at the local level. Our solution to this classification issue is to use the snapshot ensemble to improve performance. The most obvious and straightforward way of combining these snapshot models is to simply do majority voting. This allows each model to make a prediction and if over half of the models agree on one class, that is the class assigned to the image. However, we found that this method did not work well with this data and the performance actually decreased by 1 correct animal prediction compared to the single best model. Likely this is due to the individual models not being very confident in their predictions. We then looked to averaging the predictions which showed a noticeable increase in performance. The ensemble averaging procedure involves allowing each of the 11 models to make a prediction on each image within a single brain; subsequently, those 11 predictions' softmax outputs are averaged and the class with the highest average confidence is applied to that image. After all the images of a single brain are classified, the majority criteria approach classifies that brain as either high or low proliferation. Our snapshot ensemble averaging method correctly predicted the global class (high or low proliferation) of microglial cells in 78.6% of the cases (i.e., 11 of 14 correct). This result for the snapshot ensemble method is based on training with microglial cell densities in local (20x) images with all images assigned a global label regardless of individual image characteristics. Table 1 summarizes the performance of the single best model, ensemble voting, and ensemble averaging methods.

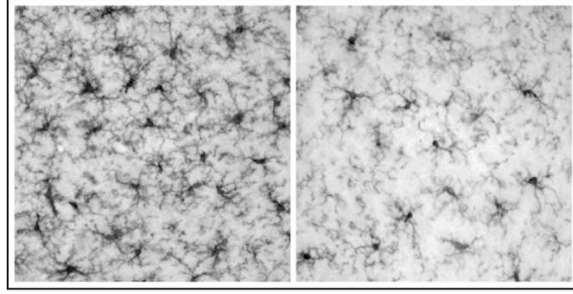


Figure 2. Left: GT Label High Density, Right: GT Label Low Density

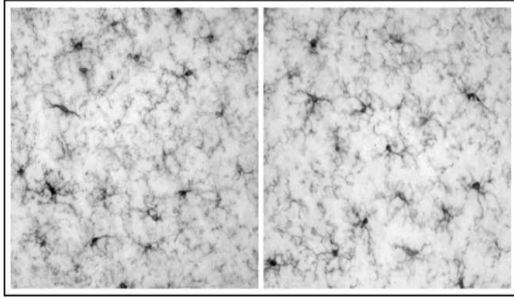


Figure 3. Left: GT Label High Density, Right: GT Label Low Density

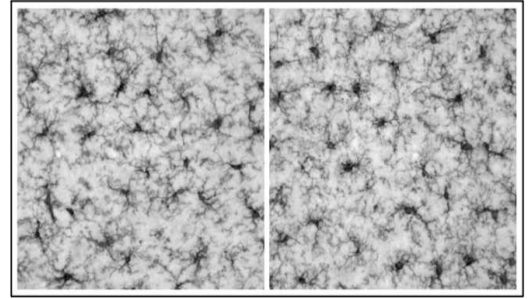


Figure 4. Left: GT Label High Density, Right: GT Label Low Density

Table 1. Results for n=14 mouse brain predictions

Method (%)	Accuracy (%)	Precision (%)	Recall (%)	F1 (%)
Single Best	57.1	55.6	71.4	62.5
Ensemble Voting	50.0	50.0	85.7	62.9
Ensemble Average	78.6	75.0	85.7	80.0

4. CONCLUSION

Current methods for assessing microglial cell proliferation (neuroinflammation) in specific regions of brain and spinal cord require an extensive degree of manual cell counting by a trained expert. As this approach is subjective, error prone, time- and labor-intensive, there is a substantial opportunity for the use of machine learning to automate this process, and thereby accelerate the rate of scientific research, medical discoveries and drug discovery related to neuroinflammation. There is some previous work on the automatic segmentation of these cells at high magnification^{2,3,14} that can be used to estimate cell counts, these methods require a human-in-the-loop in one form or another. This is the first application of deep learning to this classification problem at low magnification (20x). Here we present a method of using an ensemble of snapshots to automatically classify mouse brains as having high or low density of cells based on the classification of images at 20x magnification with minimal expert time requirement. We have shown on a novel dataset of 14 mice that our method can correctly classify $\sim 80\%$ of cases. Our method could provide researchers with quick and accurate estimates of cell density at low magnification. This approach could potentially benefit a wide variety of studies across the diverse disciplines of neuroscience where global proliferation of microglial cells in brain and spinal cord tissue could be predicted after

testing with unlabeled low magnification images of immunostained microglia cells on sections from those tissues. Ongoing work is focused on continuing to improve prediction accuracy of our deep learning model through the training with images from additional mice with low, middle, and high levels of proliferation at the global level.

5. FUTURE WORK

In this work we focused on a simple binary classification of animals as having either high or low density of microglia cells. However, in our sorting of cases we found many fall between these two extrema. Going forward we would like to experiment with more than two classes to determine if classification can be done on a more granular level. Adding more data and additional classes may also lead us to finding other architectures that are better suited for this problem. It is also to be seen if increasing the number of models used in the ensemble provides enough improvement to warrant their use. We would also like to try a traditional ensemble as opposed to the snapshot approach to determine if there is a noticeable increase in performance to justify the additional training time needed.

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Data used is publicly available at *

*tinyurl.com/20xData-USF-SRC