Comet Assay Classification for Buccal Mucosa's DNA Damage Measurement with Super Tiny Dataset Using Transfer Learning

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Maciej Huk
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Intelligent Information and Database Systems: Recent Developments

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Preface

The ongoing integration of various intelligent information, database systems and technologies—including artificial intelligence, social media, multimedia, cloud computing, and big data processing—leads to important synergy of those fields. It results in new practical solutions and applications as well as broadens the scope of theoretical analyses and approaches. But it also creates previously not considered concerns and problems, related not only to the scale but also to new functionalities of created methods and systems. This includes such issues as reliability and long-term integrity of critical applications, energy efficiency of big data storage, processing, organization, and quality assurance during the development of very complex systems. Continuous research in the fields of intelligent information and database systems provides an opportunity to identify and address those problems by suggesting and creating further solutions and technologies.

The main goal of this book is to present and intensify practical application of current knowledge and state of the art emerging in the field of intelligent information and database systems. Thus, it includes a set of carefully selected research papers presenting new methods, technologies and applications. The represented scope of topics encompasses a wide spectrum of research subjects discussed both from the theoretical and practical points of view. The book covers such fundamental aspects of intelligent information and database systems as artificial and computational intelligence, nature-inspired algorithms and paradigms, collective knowledge and ontologies, computer vision techniques, and multi-dimensional large-scale data processing. At the same time, it presents a number of interdisciplinary topics including social networks, system security, cloud computing, Internet of things, business intelligence, and biomedical data analysis.

This volume in the well-established "Studies in Computational Intelligence" series can be a valuable source of knowledge about current problems and solutions in the field. It contains a selection of 34 chapters based on original research presented as posters during *the Asian Conference on Intelligent Information and Database Systems* (*ACIIDS 2019*) held on 8–11 April 2019 in Yogyakarta, Indonesia. This eleventh edition of the ACIIDS conference was organized by the

Wrocław University of Science and Technology, Poland, together with BINUS University, Indonesia, and their partners.

The book will be a useful resource and reference for researchers and practitioners interested in increasing synergy between artificial intelligence and database technologies as well as for graduate and Ph.D. students in computer science and related fields.

Finally, on behalf of the Steering Committee, Program Committee and Organizing Committee of the ACIIDS 2019 we would like to thank all the authors for sharing their research during the conference. We also extend our thanks to all reviewers for their time, engagement, and expertise. And special gratitude we express to Prof. Janusz Kacprzyk, the editor of this series, and Dr. Thomas Ditzinger from Springer for their interest and support for our project. The help of those people was invaluable in the preparation of this book.

Yogyakarta, Indonesia
April 2019

Maciej Huk
Marcin Maleszka
Edward Szczerbicki

Contents

Comet Assay Classification for Buccal Mucosa's DNA Damage Measurement with Super Tiny Dataset Using Transfer Learning

Afiahayati, Edgar Anarossi, Ryna Dwi Yanuaryska, Fajar Ulin Nuha and Sri Mulyana

Abstract Comet assay or single cell gel electrophoresis assay (SCGE) is a method which is frequently used to measure the damage of DNA. The results of comet assay is a set of comet images, then the comet images are classified to measure the level of DNA damage. Currently, there are several softwares for comet assay image analysis, both free and commercial. Commercial software is quite expensive, while free software is limited, especially for buccal mucosa cell and super tiny comet image dataset. In this research, we propose a classification model for comet assay with super tiny image dataset which is taken from buccal mucosa cells. We propose a transfer learning based convolutional neural network (CNN) model. We have compared the transfer learning model with CNN-support vector machine (SVM) and ordinary CNN. In our experiments, we use super tiny dataset consisting of 73 images. Our transfer learning model gives an accuracy 70.5%, while CNN-SVM gives 62.3% and ordinary CNN gives 63.5%. We also compare our transfer learning model with most frequently used, free comet assay analysis software, OpenComet. Open-Comet gives an accuracy 11.5%. Our transfer learning model is promising for comet assay for buccal mucosa cell and super tiny dataset.

Keywords Transfer learning · Tiny dataset · Comet assay · Buccal mucosa cell

1 Introduction

Comet assay is an excellent method for detecting DNA damage due to its versatility and sensitivity [1]. Comets can be identified and scored by visual inspection by an expert, but this method is very time-consuming and subjective. Therefore, image

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analysis software packages were developed [2]. Several tools have been developed to analyze comet assay images. Most of the tools use geometric measuring of the comet's core and tail [3–5]. Currently, there are some softwares for comet assay image analysis, both free and commercial. Commercial software is quite expensive, while free software is limited, especially for buccal mucosa cell. Comet image from different sample sources, for instance mucosa swab and cell line, have slightly different geometric algorithm. It affects the accuracy of the data. Comet images data are hard to obtain, therefore the dataset is limited.

In this research, we propose, an artificial intelligence method specifically developed to score the comet image obtained from buccal mucosa cells. There are two main challenges for this problem domain: (1) comet images obtained from buccal mucosa cells are not like ideal cells since there is a swab activity; (2) the dataset is very tiny. One of artificial intelligence algorithms that could be used to solve image classification problems are Convolutional Neural Network (CNN). CNN is a machine learning method that is designed to process visual information [6–10]. CNN could classify an image based of the patterns learned at the training image sets. The learned patterns then could be used to classify new images. CNN has good performance with a big dataset, while our comet image dataset is super tiny. Therefore, we present a transfer learning based convolutional neural network (CNN) model to classify comet images to measure the level of DNA damage. This work is an extended version of authors' work [11].

2 Data and Methods

2.1 Comet Assay Data

The used comet assay images are cropped image from a microscope slide images of buccal mucosal cells. The total number of images are 73 images. There are 5 levels of the DNA damage. Level 0 represents the lowest damage (the best DNA) while level 4 represents the highest damage (the worst DNA). Those levels are represented as classes in our classification models. Example of comet assay's microscope slide image and samples of comet assay images for each class could be seen in Figs. 1 and 2.

2.2 Pre-processing

In this research, there are 5 stages of pre-processing that produced 2 type of datasets: (1) dataset without data augmentation, and (2) dataset with data augmentation. Pre-processing stages are as follows:

Image Size Normalization Image size normalization uses the Gaussian Pyramid Multi-Scaling Analysis [12] process to expand or reduce the size of image to get as

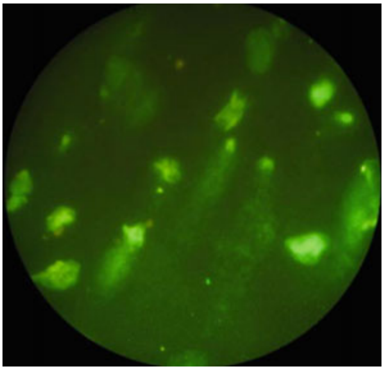


Fig. 1 Comet assay's microscope slide image

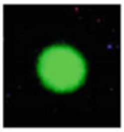
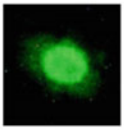
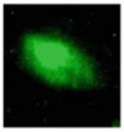
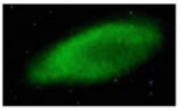
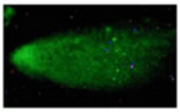
Class	*Comet Assay Image*
0	
1	
2	
3	
4	

Fig. 2 Samples of comet assay images for each class

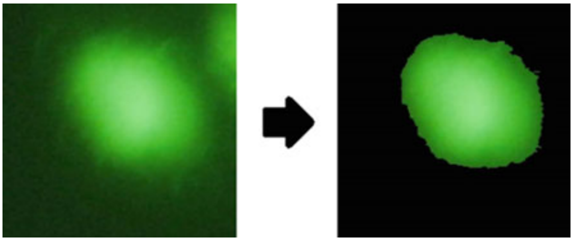


Fig. 3 Comet assay's segmentation

close to 100 × 100 pixel. After that a resize process is done using bilinear interpolation to reach 100 × 100 pixel.

Noise Data Removal Removing images from each class with a similarity percentage to other images in the same class with lower than the predefined similarity threshold. This process is used because the size of dataset are very small, so if the noise images are learned, there will be no other image that could be used to validate if the images are from the respective classes.

Image Segmentation Comet assay objects are separated from the background or other comets inside the image. This process is done to ease the machine learning model at learning the comet assay images. Segmentation is done by using threshold parameter at HSV color space. An example of the segmentation result could be seen at Fig. 3.

RGB Color Space Conversion to Grayscale Color Space Converting the image's RGB color space to Grayscale color space because for the most part of the image, only Green dimension of the RGB has positive values, meanwhile the Red and Blue dimension are zeroes.

Data Augmentation Some of the augmentations used are horizontal and vertical flipping, random cropping, random distortion, and resize. Each classes are augmented to 50 samples per class.

2.3 Classification Models

In order to get good accuracy from such a super dataset, in this research we tried several classification models that could process image data, as follows: (1) Ordinary Convolutional Neural Network, (2) CNN-Support Vector Machine, (3) Transfer Learning (our proposed method).

Ordinary Convolutional Neural Network (CNN) CNN is one of deep learning algorithm variants that is used to process 2 dimensional data. Some parameters that affects a CNN models are convolution layer, pooling layer, activation function, data being used, and how the training is done. Architecture of the CNN model used in this research is represented in Table 1 and Fig. 4.

Table 1 Specification of the CNN model

Layer	Information
Input layer	Image size 100 × 100
Convolution layer	64 filter, size 15 × 15
Pooling layer	Size 2 × 2, stride 2, 2
Convolution layer	256 filter, size 11 × 11
Pooling layer	Size 2 × 2, stride 2, 2
Dense layer	1024 neuron
Dense layer	1024 neuron
Dropout	Dropout rate = 0.2
Output layer	Softmax with 5 neuron

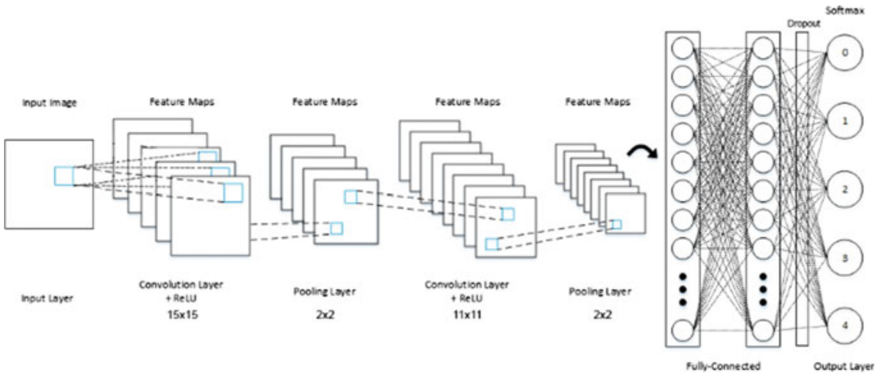


Fig. 4 The architecture of CNN model

The training process is done using the error value calculated from a loss function that is cross entropy and accelerated using ADAGRAD optimizer.

CNN-Support Vector Machine (SVM) SVM solves problems by making N − 1 hyperplane to separate data features with N feature dimensions [13]. Hyperplane or support vector is a line that separates features with as wide margin as possible. Each data will then be categorized into a class according to it's features from the hyperplanes.

Some parameters that affect SVM with RBF as kernel are C and gamma that was tuned in this research. C is a parameter that affects the magnitude of penalty given for each of the misclassified data, meanwhile gamma is a parameter that affects how far the influence of single training example reaches. Best parameters of the SVM model are obtained by using Grid Search. The range of parameters used at grid search is shown in Table 2. The input of SVM in this research is same with the input of the first dense layer at CNN model.

Table 2 Range of parameters used at grid search

Parameter	Range
C	[0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1, 1.1, 1.2, 1.3, 1.4, 1.5, 1.6, 1.7, 1.8, 1.9, 2]
Gamma	[0.0000001, 0.000001, 0.00001, 0.0001, 0.001, 0.01, 0.1, 1]

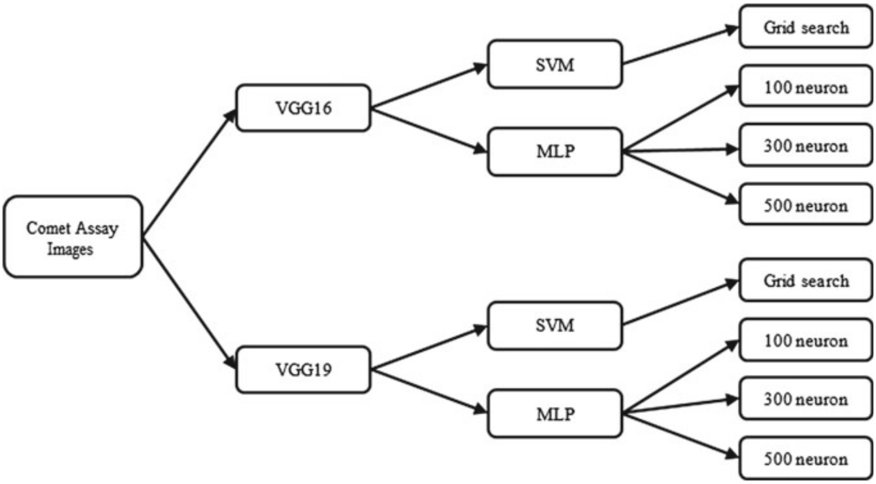


Fig. 5 Classification process using transfer learning

Transfer Learning Transfer learning or inductive transfer is a technique that focused at storing knowledge obtained from solving a problem and applying it to another problem. Transfer learning can be applied at deep learning if a feature learned by a model is general enough to be applied at a target data.

There are two kinds of approach that could be used in transfer learning, that is (1) building a model from scratch and (2) using a pre-trained model. In this research we use a pre-trained model of VGG16 and VGG19 architecture that had been trained on ImageNet dataset which includes 1000 classes [14]. To be able to use the transfer learning model's outputs at classifying comet assay, we used VGG16 and VGG19 outputs as an input at the next classifier that is Multi Layer Perceptron with 1 hidden layer or a Support Vector Machine to get the desired 5 class. Classification process using transfer learning is shown at Fig. 5.

3 Results and Discussion

3.1 Experiment Configuration

For every classification models being tested, 2 types of datasets are used, that is: (1) dataset without data augmentation which includes 61 images, and (2) dataset with data augmentation which includes 250 images. Validations are done using k-fold cross validation with a k value of 4. This research is implemented in Kubuntu 16.10. The CPU is Intel Core i7-6700 CPU @ 3.40 GHz with 16 GB of RAM and only for Ordinary CNN, we use parallel processing with GPU 1080 Ti.

3.2 Results of Ordinary CNN

For the CNN model, testing is exclusively split into 2 parts, which is: (1) testing of parameters related to the quantity of data which includes data augmentation and batch size, and (2) testing of parameters related to the training time which includes learning rate and step size.

The average time of the tests is directly proportional to the size of batch size being tested. Results of data augmentation and batch size tests are shown at Table 3. It is also shown in batch size tests that batch size affects the stability of accuracy and loss function in the training, wherein larger batch size will decrease the amount of change done for each step. From the results obtained from data augmentation and batch size tests at the CNN model, it is shown that unaugmented data has higher accuracy compared to data with augmentations. Furthermore, for the dataset with such a small number, which is $75\% \times 61 = 45$ training data, the best batch size to use in this case is 1 or we could say that the best method for this case is using online learning.

From the results of learning rate and step size experiments, represented at Table 4, it is shown that the best learning rate is 0.00001, whereas using 0.0001 results is a nonoptimal learning while using 0.000001 makes the learning process too slow and

Table 3 Data augmentation and batch size tests results

Batch size	Data	Accuracy	Average time (s)
1	No augmentation	**0.634615406**	237.25
	Augmented	0.500000015	239.5
10	No augmentation	0.519230783	291.25
	Augmented	0.442307696	285
20	No augmentation	0.557692327	291.25
	Augmented	0.519230783	291.25

Table 4 Learning rate and step size tests results

Learning rate	Accuracy (500 steps)	Accuracy (1000 steps)	Accuracy (1500 steps)	Accuracy (2000 steps)
0.0001	0.40385	0.4423	0.40385	0.403846167
0.00001	0.5	0.5577	0.538475	**0.557725**
0.000001	0.365375	0.403825	0.3846	0.403825

Table 5 Best parameter and result of SVM model in CNN-SVM

Data	Accuracy	C	Gamma	Average time (s)
No augmentation	**0.62295082**	1.1	1.00E−07	36
Augmented	0.568	1.5	1.00E−07	345

results in another nonoptimal learning. Too small of a learning rate also results of being stuck in local minima. Size of step size used in this tests isn't too relevant because the step size needed depends on the size of the learning rate.

3.3 Results of CNN-SVM

For the CNN-SVM model, it is shown that most of the tests with unaugmented data results in higher accuracy compared to tests with augmented data. For the gamma parameter, high values result in an optimal model, while small values result in an overfitting model. Furthermore, optimal C value ranges around 0.7–2, so we could say that the penalty given to each misclassification is quite small. The best results of both parameter tests are shown in Table 5.

3.4 Results of Transfer Learning

Transfer Learning model produces the best accuracy compared to the previous models. Similar to previous models, classifications using VGG16 as a feature extractor also results in higher average accuracy when using unaugmented datasets compared to using augmented datasets. From the 4 types of classifiers used to process VGG16's output, it is shown that 1 hidden layer MLP with 300 neuron results in the highest average accuracy. Results of MLP and SVM classification with VGG16 as a feature extractor is shown at Table 6.

Lastly, different from other models' results, Transfer Learning using VGG19 as a feature extractor produces higher accuracy when using augmented datasets rather than unaugmented datasets. This could be caused by 3 extra weight layers that is owned by VGG19 therefore VGG19 could store more features from more data.

Table 6 Results of transfer learning using MLP and SVM as classification methods with VGG16 as a feature extractor

Data/architecture	SVM	MLP 100	MLP 300	MLP 500
No augmentation	0.6202	**0.7049**	**0.7049**	0.639
Augmented	0.6272	0.664	0.68	0.604

Table 7 Results of MLP and SVM classification with VGG19 as a feature extractor

Data/architecture	SVM	MLP 100	MLP 300	MLP 500
No augmentation	0.6238	0.6557	0.623	0.623
Augmented	0.6356	**0.692**	0.668	0.56

Table 8 The accuracy comparison of the overall classification model and OpenComet software [15]

Data/architecture	CNN	CNN-SVM	Transfer learning 1 (VGG16-MLP)	Transfer learning 2 (VGG19-MLP)	OpenComet
No augmentation	0.635	0.623	**0.705**	0.656	0.115
Augmented	0.519	0.568	0.68	0.692	–

From the 4 types of classifiers used to process VGG19's output, it is shown that 1 hidden layer MLP with 100 neuron results in the highest average accuracy. Results of MLP and SVM classification with VGG19 as a feature extractor is shown at Table 7.

The overall model is also compared with the most frequently used, free comet assay analysis software, OpenComet [15]. The data used for OpenComet is same with data for all of our models. The results are represented in Table 8. Our transfer learning 1 model has the highest accuracy compares to other methods. Our transfer learning model gives an accuracy 70.5%, while CNN-SVM gives 62.3% and ordinary CNN gives 63.5% and Open-Comet gives an accuracy 11.5%. CNN model trained with the original comet assay's data gives lower accuracy if compared to transfer learning model that is trained using other dataset (ImageNet), which proves that kernel learning as a feature extractor in Convolutional Neural Network could be done using other dataset with no correlation to the target dataset [16].

4 Conclusion

In this paper, we have classified comet assay images from buccal mucosal cells with super tiny image dataset using several machine learning-based classification models. The CNN model trained with the original comet assay's data gives lower accuracy than the transfer learning model trained with other dataset. Kernel learning as a feature extractor in CNN could use features obtained from learning other dataset at the target dataset, so transfer learning could be used at cases where the target dataset size is too small. Our transfer learning model has the highest accuracy compares to other methods. Our transfer learning model gives an accuracy 70.5%, while CNN-SVM gives 62.3% and ordinary CNN gives 63.5% and Open-Comet gives an accuracy 11.5%. Our transfer learning model is promising for comet assay for buccal mucosa cell and super tiny dataset.

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