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Triplet loss for Chromosome Classification

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Abstract

The analysis of chromosomes, known as karyotyping, is essential in diagnosing various human genetic disorders and chromosomal aberrations. It can detect a variety of genetic diseases and provide a deeper insight into the human body. However, the process of manual karyotyping is highly time-consuming and requires accomplished professionals with a deep understanding in the field. An automated process is thus highly desirable to assist cytogeneticists and mitigate the cognitive load procured during karyotyping. With that intention, a similarity learning approach is proposed in this paper using 'Triplet Loss' for procuring high-dimensional embeddings. The Offline Triplet Loss, Semi-Hard Online mining, and associated hyperparameters are thoroughly tested and explored, and the obtained embeddings are used to classify the images into their respective chromosome classes and Denver groups. A comparative analysis on various embedding-classifying algorithms such as Multi-Layer Perceptron (MLP) and Nearest Neighbours is also demonstrated in this paper, along with experiments on associated distance metrics. The proposed methodologies deliver a superlative performance when compared to a baseline Convolutional Neural Network (CNN), on a publicly available chromosome classification dataset.

Keywords: Deep Learning, Triplet Loss, Karyotyping, Siamese Network, Image Classification

1. Introduction

Chromosomes are thread-like structures present in the nucleus of a cell and contain the genetic information of an organism known as DNA. They are the building blocks of an organism whose analysis can provide greater insight into the human body. Typically, a human cell has 23 pairs of chromosomes, consisting of 22 pairs of autosomes and one pair of sex chromosomes (XX and XY). Karyotyping, the technique of coupling and identifying the human chromosomes from cell images is used to analyze chromosomes, which provides a

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genome-wide photograph of an individual’s chromosomes and can help in providing valuable diagnostic information for specific congenital anomalies or genetic disorders like Trisomy 21 (Down Syndrome) [1] and Cancer [2]. The Giemsa staining process is used to observe the condensed chromosomes under a light microscope when the cell division reaches the metaphase stage. The distinct bands of light and dark colour created in the staining process assist in distinguishing the chromosomes [3].

Apart from the tedious and time-consuming nature of this process, highly proficient doctors in the field of cytogenetics are required to diagnose the defects and abnormalities from a karyotype image. Most of the automated karyotyping methods used for chromosome classification still require human intervention and expertise as seen in Ikaros [4], CytoVision [5] [6] [7] and ASI HiBand [8]. This paper aims to eliminate these predicaments by a deep learning implementation.

Recent advances in the field of Machine Learning have led to the achievement of more accurate results and classifications [9][10][11][12]. However, Deep Neural Networks require significant data, and thus, can be computationally expensive. A neural network trained on Triplet Loss generates a similarity learning model and produces required results with less training data. The proposed model displayed better results than a baseline Convolutional Neural Network (CNN) [13].

2. Related Work

The conventional and sophisticated statistical methods used for chromosome classification [14] do not deliver accurate results. Contrarily, artificial intelligence-based methods have shown significantly better results in chromosome classification. This paper has a classification algorithm based on the Siamese Network [15] in which the chromosomes are transformed into the input for the neural network using two approaches.

A Multi-Layer-Perceptron (MLP) classifier exploited the embeddings obtained using a Siamese Network for classification and achieved an average accuracy of 85.6%.

A CNN based deep learning network was used in the paper [16] to classify single chromosome images into 23 classes, where sex chromosome types X and Y were considered the same class, and the proposed model achieved a classification accuracy of 92.5%.

Varifocal-Net was postulated in [17] for simultaneous categorization of chromosome's type and polarity using deep convolutional networks, and a Global Scale Network (G-Net) and a Local Scale Network (L-Net) for method implementation.

An incremental learning method was proposed in [18] to classify chromosomes into seven groups based on the Denver group classification standard and yielded an overall average classification accuracy of 97%.

The paper [19] proposed a Multi-Distributed Generated Advertising Network (MD-GAN), that enhanced the training dataset by generating training samples, trained a CNN model based on the obtained samples and tested on a self-collected dataset.

The paper [20] used a deep learning network containing proxies with a Convolutional Block Attention Module (CBAM) and having ResNeXt-50 as the backbone network, which achieved a classification accuracy of 95.86%.

2 Triplet Loss approaches are explored in this paper using high-level embeddings that are utilized in accessing the classification accuracy of K-Nearest Neighbors (KNN) [21] and Multi-Layer Perceptron (MLP) [22] of the images into the respective chromosome types and Denver groups [23]. The proposed methods supersede the preceding approaches due to the ability to work for small datasets and the improved results when compared to single image input networks.

3. Proposed Work

The first of the two approaches [24] implemented in this paper is an offline Triplet Loss method, where the base network is replicated three times (with shared weights) to produce high-level embeddings of anchors, positives, and negatives, based on which the triplet loss gets calculated.

The second approach is an online Triplet Loss method, where the triplet loss is determined using semi-hard mining.

3.1 Embedding Network

The base network used for obtaining high-dimensional embeddings for karyotype analysis is called the embedding network. The neural architecture consists of four convolutional blocks, followed by one flattening layer and one fully-connected dense layer containing 1024 neurons and Rectified Linear Unit (ReLU) activation [25]. The desired embeddings are obtained by feeding the output vector into the succeeding fully-connected dense layer consisting of 128 units with no activation function. Figure 1 illustrates the schematics of the network.

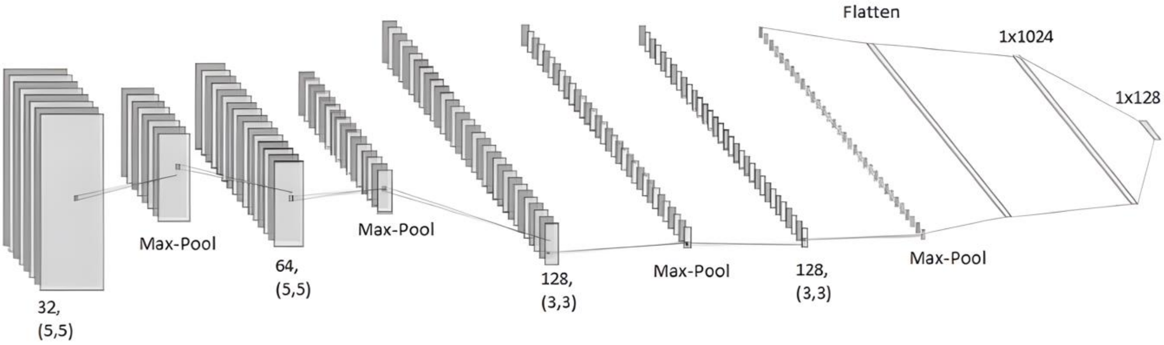

Figure 1. Schematic diagram of the embedding network.

Each convolution block alternates between a convolution layer and a max pool layer. Each convolutional layer has a ReLU unit that operates on a stride of 1 and unit dilation rate [26], and each max pool layer [27] has a pooling window of 2x2, which halves the input dimensionality and provides an abstract form of representation whilst reducing the number of parameters and computational needs. The first block of the convolutional layer contains 32 filters with a kernel size of (5,5), while the second block contains 64 filters with a kernel size of (5,5). The third and the fourth blocks contain 128 convolutional filters with a kernel size of (3,3) each.

3.2 Offline Triplet Loss

A Siamese network with three input layers is trained with a loss function computed using a metric between the representations learned at the last dense layer. The three networks gain the same transformation since they have connected model parameters, making the anchor and the positive, mapped closer to each other and having a significant distance between the anchor and the negative, in the learned feature space. Figure 2 shows the schematics of this model.

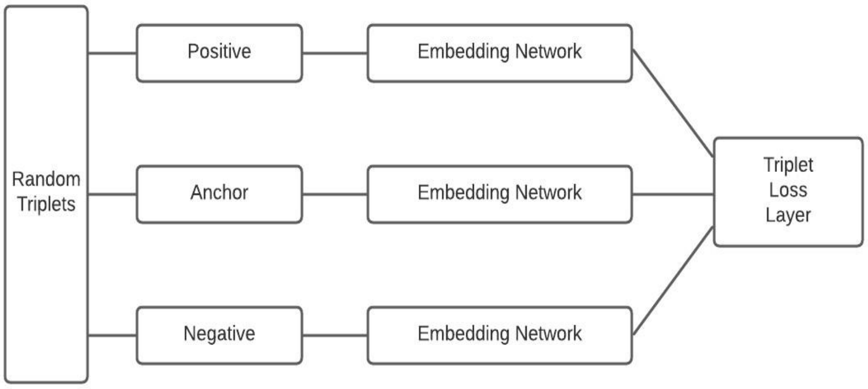

Figure 2. Block diagram of the offline triplet loss model.

Random triplets are generated by three images fed to the embedding networks, where the embeddings are further used to calculate the triplet loss.

The loss function can be defined as:

$$L(A, P, N) = \max(|| f(A) - f(P) ||^2 - || f(A) - f(N) ||^2 + \alpha, 0)$$

Here, the anchor, positive and negative images are denoted as ‘A’, ‘P’ and ‘N’ respectively. The embedding network is referred as a mathematical function ‘f’, and the margin value is kept as ‘0.2’. The inter embedding distance $((\| f(A) - f(P)\|^2), (\| f(A) - f(N)\|^2))$ is the length of the line segment connecting the two points which are calculated by assuming a coordinate system of ‘128’ dimensions and the squared Euclidean metric.

The loss function is optimized by using the Adam optimization algorithm [28], an extension of the stochastic gradient descent, due to the low computational needs and the invariance to diagonal re-scaling of gradients. To avoid an inadvertent increase in gradient descent and to maintain a decrease in training error, a learning rate of 10^{-4} is used, which provides an outstanding result. The model is trained by generating batches of triplets selected randomly from the training set as 100 per step for 115 steps per epoch for a total of 20 epochs.

The offline method needed a Siamese network for generating random triplets and did not consider their subcategories. In online mining, however, necessary triplets are computed on the fly for each batch of inputs, where T embeddings are generated for a given batch of T chromosomes, resulting in T3 triplets. The majority of the obtained entities are invalid as they do not follow the required format of an anchor, a positive and a negative triplet. Only the valid triplets are further sub-categorized using the following energy function:

$$L = \max(D(A, P) - D(A, N) + \text{margin}, 0)$$

Here, the margin is a positive constant with unit value, D symbolizes the pairwise distance between the embeddings calculated by the distance matrix and the squared Euclidean norm, and the anchor, the positive and the negative images are denoted by A, P, and N respectively.

Semi-hard triplets are used for training the model in this paper. The proposed model contains the same embedding model and hyperparameters related to the network as its offline counterpart.

The high-level embeddings are L-2 normalized and used for calculating the triplet loss. The training and testing data are divided into batches of 32 images each. Eligible triplets for each batch have two chromosome images of the same labels and one negative image, where the embeddings follow an orientation and are analogous to that of a semi-hard negative.

The loss function is optimized by the ‘Adam optimization technique’ with a learning rate of 10^{-3}. The model is trained for 30 epochs (each epoch symbolizing one forward pass and one backward pass over the entire training set). Since the training dataset contains 3347 image arrays and 32 batches, each epoch accommodates 105 steps.

3.3 Classification Multi-Layer Perceptron (MLP)

The embeddings obtained from both the online and offline loss models have a dimensionality of 128 and are used for the classification of images using Multi-Layer Perceptron (MLP) with two ReLU activated hidden layers incorporating 512 and 1024 units in the same order. The output fed into a SoftMax layer returns a probability distribution of the 24 karyotype classes where all the outputs are in the range [0,1] and aggregate to 1. The maximum value of the 24 layers is selected as the chromosome class.

There are 24 classes in this model and the Categorical Cross-Entropy (CE) loss function [29] is used to evaluate the performance during each iteration. AdaMax Optimization [28] is used to optimize the loss function with a learning rate of 10^{-3}, giving superior performance than 'Adam' for training embeddings due to the former's sparse behavior in contrast to dense behavior.

3.4 KNN (K-Nearest Neighbors)

An alternative approach to classify embeddings is the K-Nearest Neighbors (KNN) algorithm and is assessed by testing different distance algorithms like Manhattan distance (L1 norm), Minkowski distance metric [30] (unit p), and Euclidean distance (L2 norm). An absolute difference of coordinates is used in the case of Manhattan distance instead of Euclidean distance to avoid the curse of dimensionality as the L1 norm surpasses the default L2 norm for higher-dimensional classification tasks [31].

3.5 t-SNE Visualization

The improvement in classification of the proposed model can be explained by their t-distributed Stochastic Neighbor Embedding (t-SNE) visualization [32], which converts similarities between the data points to joint probabilities, and aims to minimize the Kullback - Leibler divergence [33] between the joint probabilities of the resultant t-SNE pairs and the high-level embeddings from the proposed models. Though t-SNE is computationally expensive, it outperforms conventional linear dimensionality reduction algorithms like PCA [34] by minimizing the inter-embedding distance of homogenous data points and maximizing inter-cluster, whereas PCA is only successful in processing the latter.

4. Dataset

The dataset consists of 4184 images of standard chromosomes of size 220 x 100, taken from a publicly available dataset [35] having 91 different karyotype images. The preprocessing involves the conversion of chromosome images into grayscale and normalization in the range [0,1] by dividing the grayscale value of each constituent pixel by the highest number possible i.e., 255. No further data augmentation or preprocessing is applied. 80% of the data is selected randomly for training, and the rest is reserved for testing the model’s classification accuracy. The same train-test split is used to differentiate the model's performance between the baseline networks and the triplet loss models.

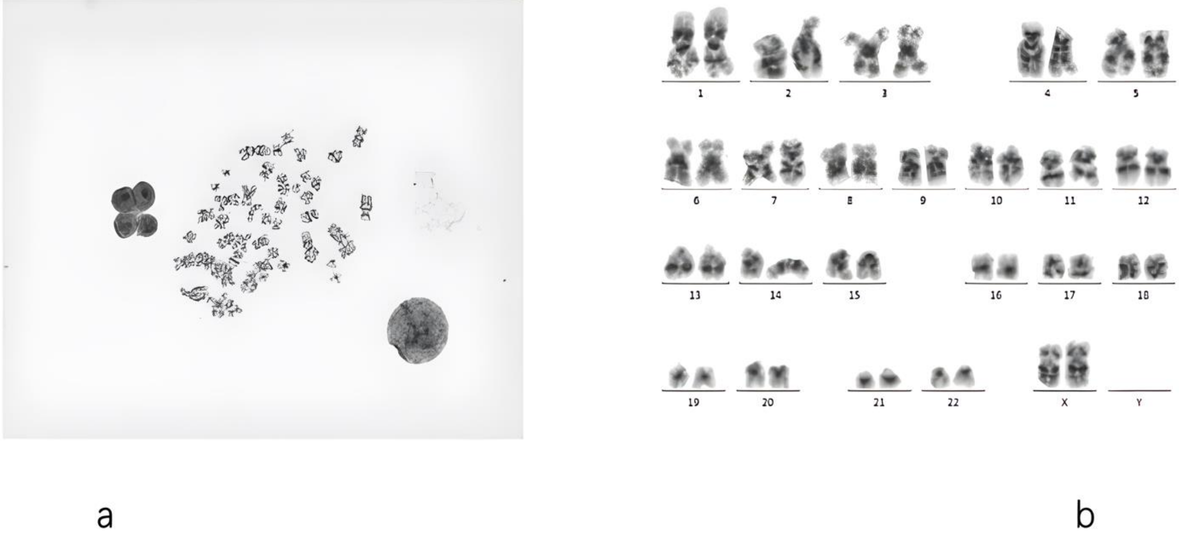

Figure 3. A sample of chromosomes in a normal female cell and its corresponding karyotype [35].

5. Results and Discussions

This section contains the results regarding the deep learning implementations. The results from the proposed models are validated and compared to an MLP trained on the flatten images and a baseline CNN. The baseline MLP has three ‘ReLU’ activated layers of size 128, 64 and 32 which are followed by a SoftMax layer. The model is trained with ‘Adam optimizer’ and the categorical cross entropy loss function with a learning rate of 10^{-3} for ‘25’ epochs.

The baseline CNN consists of three convolutional blocks, each embodying a ‘ReLU’ activated convolutional layer operating on a stride of ‘1’ and unit dilation rate, a max pool layer of window size (2 x 2) and a dropout layer with a dropout rate of ‘0.35’. The convolutional layer in the first block has 32 filters with a kernel size of (7,7), the second block contains 32 convolutional filters with a kernel size of (5,5), and the third block contains 64 convolutional filters with a kernel size of (3,3). The convolutional blocks are followed by a Flattening layer, a dense layer with 128 neurons, a ‘ReLU’ activation layer and a SoftMax layer, which gives the probabilistic distribution of the input image with the 24 chromosome classes. The network is implemented with the categorical cross entropy loss function and ‘SGD’ optimization (Stochastic Gradient Descent optimizer) [36] with a learning rate of 10^{-2} for ‘45’ epochs.

The obtained results are tested for accuracy in classifying both, the correct chromosome class and the Denver group as shown in table 2. The existing predictions of the 24 classes are used to obtain the predicted Denver group. The following relation is used to convert the predicted classes [37].

Table 1. Chromosomes classes corresponding to their Denver groups

Denver Group	Chromosome Classes
Group A	#1-#3
Group B	#4-#5
Group C	#6-#12, X
Group D	#13-#15
Group E	#16-#18
Group F	#19-#20
Group G	#21-#22, Y

Table 2. Classification accuracy for Chromosome classes and Denver Groups of various Network Architectures.

Network Architecture	Classification Accuracy (24 chromosome classes)	Classification Accuracy (Denver Groups)
Baseline MLP	40.262	48.262
Baseline CNN	79.928	81.600
Offline loss + KNN (E)	89.605	91.158
Offline loss + KNN (M)	89.964	91.397
Offline loss + MLP	87.335	89.605
Online loss + KNN (E)	88.052	89.486

Online loss + KNN (M)	88.172	89.725
Online loss + MLP	88.052	89.844

The results regarding the proposed models are displayed in rows 3 to 7. The offline loss model with KNN using the Manhattan distance norm outperforms the baselines for the chromosome classification and the Denver group classification with a significant margin of 49.702% and 43.135% for the MLP, and 10.038% and 9.797% for the CNN.

The total training time for the online triplet loss model aggregates to 51.419 seconds, consuming an average of 16.323 milliseconds per step.

The offline triplet loss model averages 0.00103 seconds per triplet, aggregating 238.286 seconds of total training time. While the offline loss models display more accuracy, the online loss models show reduced training time with comparable accuracy. However, both the models are proved superior compared to the control networks.

The use of Manhattan distance instead of Euclidean distance in the KNN classifier can also be deemed successful as the KNN implementations, for both the online and offline loss models have an increase in classification accuracy of the chromosome classes and the Denver groups by 0.120% and 0.119%, and 0.356% and 0.239% respectively. KNN algorithms yield preferable performances with a slight increase in accuracy regardless of the distance metric compared to a MLP classifier due to the favorable hyperspace generated by the Siamese encoder.

5.1 Embedding Visualization

Figure 4 displays the two-dimensional description of the high-dimensional embeddings resulting from the scatterplots generated by the t-SNE algorithm.

The t-SNE scatterplots of the offline and online losses are produced using the embeddings of size 128. The resultant embeddings are compared with the testing data using the t-SNE plot for the test dataset created from the flattened image arrays of size 22000. Figure 4 makes it clear that the use of triplet loss models minimizes the intra-class embedding distance whilst maximizing the inter-class embedding distance, resulting in an increased classification accuracy.

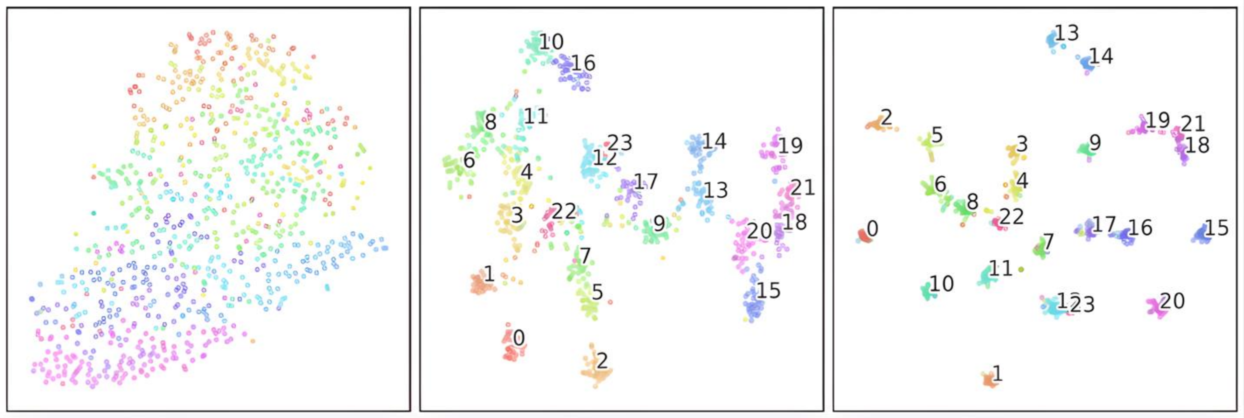

Figure 4. t-SNE scatterplots for the original testing set, the embeddings from the offline loss model, and the online loss models with the t-SNE cluster centers

The Keras API [38] is used for implementing the deep-learning methods and the Sci-kit learn framework [39] is used for the KNN algorithms and t-SNE embeddings implementation.

6. Conclusion and Future Work

Karyotype images are a source of significant diagnostic information, and understanding individual chromosomes and analyzing karyotypes may assist in recognizing many genetic defects. However, experienced professionals are required to achieve promising results. With the above-mentioned causes, this paper motivates a unique deep-learning approach capable of providing a highly accurate and quick results. Instead of a conventional CNN, a triplet loss model is trained for obtaining high-level embeddings, and the embeddings are further used for classification. The proposed models showcase excellent performance compared to a standard CNN, with a chromosome classification accuracy of 89.964% and a Denver group classification accuracy of 91.397%. The results are achieved without any data augmentation and all the models are trained with identical train-test splits. In professional tasks, data augmentation has proven useful; as embeddings are used for classification, the proposed models permit the use of unconventional augmentation methods. Looking ahead, it can be said that, instead of augmenting the images, proven methods like E-Mixup, E-Stichup, and Soft E-Stichup [40] can be used to augment the embeddings from the proposed models. A combination of triplet loss models can also be trained to emphasize each aspect of a chromosome and a concatenation of the resultant embeddings can be used for further classification.

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