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Chromosome Karyotyping and Aneuploidy Detection using Deep Learning Networks

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Abstract

Chromosome karyotype analysis is a vital role in the diagnosis of congenital disease, chromosomal aneuploidies. Karyotyping is defined as the process of categorizing chromosomes into one of 24 kinds. Chromosomal aneuploidies can be classified as structural and numerical. Numerical anomalies such as Down syndrome, Williams's syndrome Pattu syndrome, Turner syndrome, klinefelter syndrome and certain kind of cancers detected with the help of karyotyping. It necessitates rigorous attention to detail and well-trained people. With these objectives in mind, in this work, we automated karyotyping and identified common numerical aneuploidies using deep learning on a dataset of 5474 distinct grayscale metaphase chromosomal impressions from the Bioimage chromosome categorization dataset (BioImLab), which is freely available online. Three stages are included in the proposed classification model. The first stage entails segmenting and individual chromosome identification using bounding box detection and modified binary mask methods. At this point, achieved individual chromosome detection accuracy is 99%. The second stage involves classifying chromosomes using a 144 deep and 1000 FC layer GLNet (GoogleNet) classifier, which has an accuracy rate of 96.33%. The last step is to accurately identify aneuploidies with a 98% detection rate. A robust automatic classification system has been developed. Many scholars have proposed various karyotyping approaches. This research compares and contrasts the outcomes of several approaches with greater accuracy.

Keywords: Chromosome Karyotype, Deep learning, GLNet classifier, Aneuploidy Detection.

1.Introduction

In cytogenetic labs, chromosome karyotyping [1] is often used as a screening and diagnostic tool. Chromosomes are dyed with a fluorescent protein before being photographed via a microscope for examination and categorization. Every chromosome in the picture must be recognized and allocated to one of twenty-four classes; the result is a 'karyotype image,' wherein chromosomes are visually organized according to the ISCN categorization [2].

A typical Phase Alternating Line Resolution Q banding Typical Pro-metaphase image and the accompanying Manual karyotyping of the Chromosomes are shown in Figure 1a and 1b. The majority of work aimed at developing methods for banded chromosomal analysis are focused on metaphase chromosomes, minimizing segmentation issues caused by contacts and overlaps in the later pro- metaphase. Image enhancement, features extraction, thresholding, disentanglement of group of chromosomes, segmentation, and classification are all phases in the traditional automatic karyotyping system framework.

Several strategies were also used to improve classification accuracy. Several chromosomal properties have been considered for classification: some (e.g., area, relative density, and convex hull perimeter) are directly determined from the picture, while others (e.g., chromosome axis or polarization) are computed after the chromosome has been polarized [5].

Another method [6] for profile extraction is based on looking at the contour's important points and changes. [7] suggests a way to show the shape of chromosomes using the wave packet transform. The length, centromeric index, and density profile are the key distinguishing criteria for categorization, according to [8]. The MLP trained with a back propagation neural technique is the most prevalent classifier used in literature. Recent research has also shown that density profile can only be used for accurate classification, for example, using a correlation profile classifier to perform sub - set matching over a regularly updated dataset [10], or constructing a classifier [11] based on adaptive time warping, which would have the advantage of requiring a much smaller training set than traditional methods.

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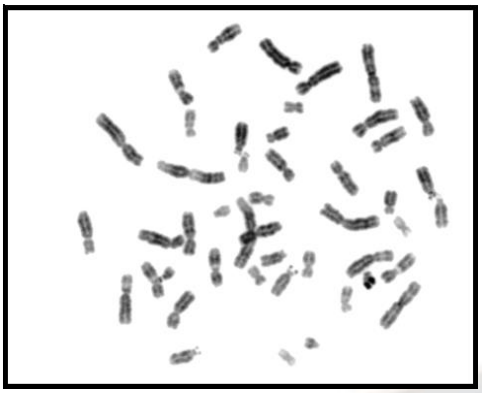

Fig. 1a. Typical Pro-metaphase Image

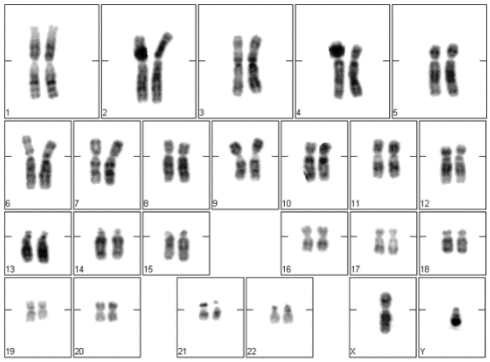

Fig. 1b. Manual karyotyping of the Chromosomes

2. Review of Chromosome Classification for Abnormality Detection

Several research works have been done in chromosome classification. In Table I numerous image processing techniques have been discussed, as well as their benefits and drawbacks. In 2021, For denoising G-banding chromosomal pictures, Emrecan Altinsoy [1] used a cascaded NN architecture. There are two steps to this procedure. First, we construct the segmentation network using the U-net and residual units. The classification block is the second phase, which is used to optimize the denoising procedure and minimise pixel losses on the genomes. 98.74 % accuracy is also achieved.

In 2020, Chengchuang Lin [2] extracts and classifies geometric information such as object area, major axis length, bounding box length, perimeter, convex area, and using various machine learning techniques, achieving an AUC of 96.21 % accuracy. Python was used to implement this.

In 2020, Mona Salem Al-Kharraz [3] used deep learning to karyotype with 147 non-overlapped metaphase images. Karyotyping achieving by three steps. First step is detection of individual chromosome using YOLO V2 convolutional neural network. The chromosomal categorization using VGG19 is the next step. The third phase is abnormality identification, which is based on the classification results. and it obtained 96.67% detection accuracy.

An expanded Residual Network was initially developed by Chengy U Wang in 2020 to isolate characteristics from images of individual chromosomes [4]. On the basis of a test dataset, a label feature vector recovered for each of the 24 chromosomal types. One of the 24 feature vectors used as labels was compared to the feature vector used to describe the input image via hausdorff distance. To reduce label space and improve chromosomal classification efficiency, a Label Redistribution technique was adopted. The classification accuracy of proposed method is 94.72 %.

Hua Bai presented a deep learning-based chromosomal extraction method in 2020 [5]. The original micrographs were segmented using U-Net to eliminate backdrop noise like nuclei and various types of interferences. Then, using YOLOv3, each chromosome was detected and extracted. The classification accuracy of proposed method is 99.3 %.

Two Deep -CNNs (Convolutional Neural Networks) for chromosomal categorization are modelled in Remya R S, 2020[6]. A preliminary investigation of these models' hyperparameters has been carried out. For chromosomal categorization, several cutting-edge CNN models are tested and assessed. The performance of all of these Deep-CNN models is compared in order to establish ideas about key parameters for efficiently classifying chromosomes.

Yulei Qin,2019 is made up of both Global-Net and Local-Net. There are three steps to it. The initial procedure is to become familiar with the both G-Net & L-Net characteristics. Using the G-Net, we extract global characteristics and discover local areas. Extract local characteristics using the L-Net by suggesting a varifocal technique. In an examination of 1909 karyotyping cases, the recommended VF-Net achieved the highest perfection per individual instance (percent) of 99.2.

Ning Xie and colleagues [8] offer mCNN GO, an integrated workflow for autonomously segmenting and classifying chromosomes using a combination of CNNs with many inputs and geometry optimization. Mask Region-Based Convolutional Neural Network was used to separate genomes from metaphase chromosomal pictures, and m Convolutional NN GO was trained to categorise the auxiliary-images. To assure the uniformity of the data and the 91.67 % accuracy attained, a geometric method was devised to straightening the chromosomes before categorization.

2019 [9] Siew Chin Neoh Prediction of Down syndrome based on three factors 1. Isolation forest technique for pre-judgment, 2. Voting strategy for model ensemble, 3. Logistic regression approach for final judgement. Important input features, Alpha – Feto protein, Human chorionic gonadotropin, un conjugated estriol has been extracted. Accurate diagnosis for Down syndrome can be achieved with accuracy TPR- 95%, FPR – 4%.

In Kiruthika P, 2018 [10] proposed a deep learning algorithm based on convolution to categorize chromosomes for autonomous karyotyping and feature extraction done by rectified linear unit (ReLU). For sex chromosome 100% accuracy obtained and implemented with keras-tensorflow.

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In Monika Sharma, 2018 [11] Proposed Res-CRANN the Res-CRANN uses this characteristic of band sequence to classify chromosomes.

Res-CRANN is a from start to finish trainable system in which a set of feature vectors are fed into Recurrent Neural Networks (RNN) from feature maps made by Res Net's convolutional layers then an awareness mechanism is tried to apply in addition to RNN sequences of outcome, they are then categorized among the 24 categories. Proposed method obtained 3% Top-1 classification accuracy.

In Swati, 2018 [12] proposed after converting low-resolution chromosomal photos into high-resolution images, a Super-Xception network brands them to one of the 23 categories based on their chromosomal makeup. Incorporate High-resolution deep learning model with traditional classification systems in this network, such as the Xception structures in our scenario.

The structure is trained from beginning to end, with super-resolution layers assisting the process of turning low-magnification images into high-magnification ones.

In Neethu Sathyan M, 2016 [13] which chromosomes are classified in to Denver class based on morphological features. Region labeling algorithm used for segmentation,GLCM used to extract texture features and ANN used for classification.

In Faroudja Abid,2016 [14] used Medial axis transform (MAT) to extract medial axis, chromosome length, centromere index for ANN classifier to chromosome classification.

In Prachi Joshi 2012 [15] autonomous karyotyping of metaphase chromosomes, a novel incremental learning technique for chromosomal categorization was developed.

It uses knowledge accumulating to categorize the chromosomes into Denver groups (A–G) and handles the problem of catastrophic fading with the creation of additional classes. The suggested method's adaptive nature helps to its long-term accuracy, even for constantly changing data.

In Mousami V. Munot, 2012[16] presented an effective method for automatically pairing chromosomes based on assessing their propinquity. The N-factor, a new Nearness Factor, is used to pair chromosomes based on band pattern similarity.

The algorithm's performance was evaluated and assessed using 50 photos from a publicly available data set of group 'A' and group 'C,' yielding an aggregate pairing accuracy of 100% for group 'A' and 97 percent for group 'C.'

3. Comparative Analysis of Classification of Chromosomes

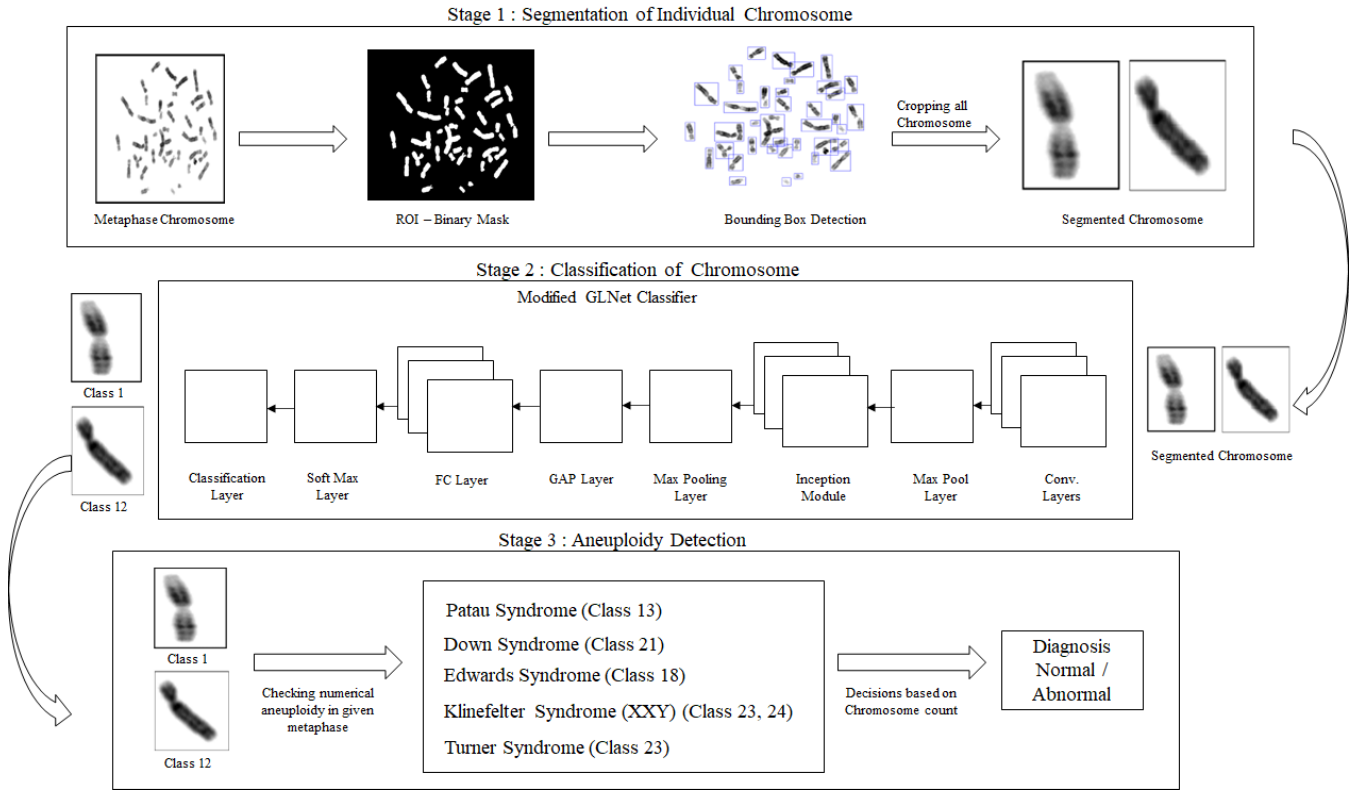

Fig. 2. Proposed system framework

S.No	Authors	Methods			Advantages	Accuracy
		Segmentation	Feature Extraction	Classifier		
1	Emrecan Altinsoy,2021[1]	U-NET & Residual Units.		Cascaded CNN(Denoising), Binary Network.	Achieves higher Dice score and removes Background noise, blotches & non-chromosome objects.	98.74%
2	Chengchuang Lin,2020[2]		Geometric features are extracted. (Object Area, Bounding Box Area, etc)	Different Machine learning Algorithms.	Higher accuracy achieved in classification of Genetic Disease.	96.21% (AUC) Python
3	Mona Salem Al-Kharraz, 2020[3]	YOLOv2 ConvolutionalNeural Network	ResNet50	CNN-VGG19 network	Accuracy is improved with ResNet50 and VGG 19.	95.04%
4	Chengy U Wang, 2020[4]		Extended ResNet		Hausdrff distance between feature vector and twenty-four label feature vectors were calculated. To minimize label space, a label redistribution approach was applied.	94.72%
5	Hua Bai,2020[5]	U-NET		YOLOV3	Achieves highest accuracy even in overlaps and adhesions.	99.3%
6	Remya R S,2020[6]			Deep CNN	Hyper parameters are computed.	
7	Yulei Qin,2019[7]		G-NET, L-NET, Residual & Multitask Learning.	Multilayer Perceptron	Improved accuracy by selecting the best features.	99.2%
8	Ning Xie,2019[8]	Mask R CNN		mCNN_Go		91.67%
9	Siew Chin Neoh, 2019 [9]	Isolation forest method.	Model Ensemble by voting scheme.	Logistic Regression.	Accurate diagnosis for Down Syndrome can be achieved.	TPR- 95% FPR – 4%
10	Kiruthika P,2018[10]		Rectified Linear Unit (ReLU)	CNN		100% (Sex chromosomes) Keras with Tensorflow-Python

11	Monika Sharma, 2018[11]	Preprocessing-Length Normalization.	Feature Vector by ResNet.	Res- CRANN	Gives higher classification accuracy.	3% Top 1 classification accuracy.
12	Swati, 2018[12]	-	-	Super xception network	Low magnification chromosome images converted to high magnification chromosome images using convolutional super magnification layer.	92.36%
13	Neethu Sathyan M, 2016[13]	Region labelling algorithm.	Morphological features, Texture features extracted by GLCM	ANN	Gives a greater outcome for individual chromosomal classification. .	Classification accuracy is 75%.
14	Faroudja Abid,2016[14]		Chromosome length, centromere Index (CI), Medial axis using MAT.	ANN	Increased accuracy in detecting anomalies and diagnosing genetic illnesses.	92%
15	Prachi Joshi 2012[15]		Chromosome length,Arm ratio, Centrometric index.	Incremental Learning.	Highest classification rate achieved by Incremental learning.	97% with 1800 chromosomes
16	Mousami V. Munot, 2012[16]	-	N-factor (Nearness factor)	NN	Determines the closeness between chromosomes in order to produce consistent results when pairing chromosomes.	Accuracy for Group A-100% Group C-97%.

4. Process Steps for Chromosome Classification

The classification process is divided into three stages. Individual chromosomes are first identified using bounding box detection and modified binary mask techniques. The separated chromosomes are the results of the first stage [1]. Chromosome classification, the next phase, use the previously separated chromosomes as input and generates classified chromosomes as output using a 144 deep and 1000 FC layer GLNet (GoogleNet) classifier. Aneuploidies identification, the last phase, is dependent on the classification stage's findings. The stages are described in detail in the sections that follow. Figure 2 depicts the suggested system in its entirety

4.1 First Stage: Individual Chromosomes Detection

We used object recognition with deep learning to find the presence of chromosomes depicted in Figure 3 and place a bounding box around each one. Metaphase Chromosome images are given to the ROI based Binary Mask detection block where flood fill operation is used to get binary mask of respective image shown in Figure 4. Adaptive and area filter is used to removes irrelevant artifacts from output image. Filtered image applied to bounding box detection block, where Image labeling and regionprops functions are used to detect bounding box. It predicts boundaries using characteristics from the entire picture. Each of the cells in the input picture is capable of estimating class probabilities, (BB) bounding box positions, (BB) confidence scores During training, well before bounding boxes (BB) with appropriate Shapes and dimensions are established and shown in Figure

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5. by using imcrop operation individual (BB) bounding boxes with chromosomes are stripped shown in Figure 6

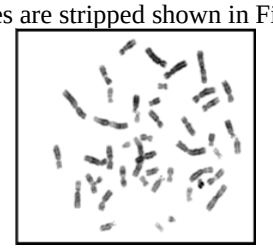

Fig. 3. Metaphase Chromosome Image

Fig. 4. ROI Based binary Mask

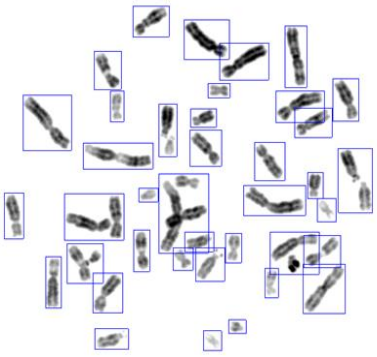

Fig. 5. Detected Bounding Box

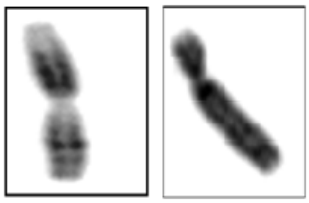

Fig. 6. Stripped Chromosome

4.2 Second Stage: Chromosome Classification

The segmented chromosomes serve as the input to the Modified GLNet at this juncture. Layers of modified GLNet are used to feature extraction and classification. The result of this phase is classified chromosomes. Modified GLNet is used as the classifier. GLNet (GoogleNet) architecture is one of the first to introduce the inception module, which drastically reduced the number of trainable network parameters. The proposed GLNet architecture differs significantly from prior state-of-the-art architectures. This architecture employs Rectified Linear Units (ReLU) as their activation functions for images of size 224 by 224 by 3 with RGB colour channels. GLNet employs 126 inception modules with 144 layers, 7 max pooling layers, 7 convolution layers, and 1 global average pooling layer. (GAP). During the training process, a fully connected classifier is applied to each layer to address the issue of vanishing gradients. The softmax classifier is given a dropout ratio of 0.4 and 6.9M total learnables.

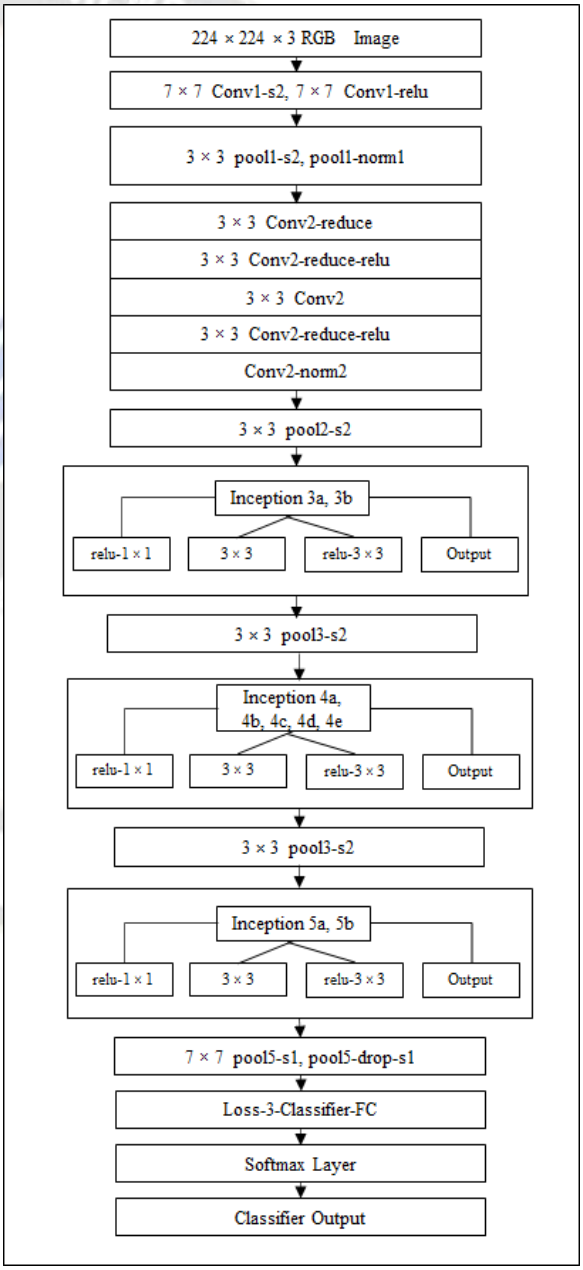

Fig. 7. Architecture of proposed GLNet

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The Figure 7 Architecture of proposed GLNet depicts the convolutional layers, inception modules, and GAP layer devised for GLNet

4.3 Third Stage: Aneuploidy Detection

The conclusion of this paradigm is the diagnosis of the aneuploidy. This stage receives classified metaphase chromosomes. The decision is based on the chromosomal count outlined in Table and is depends on a standard conditional sentence [3]. It starts with figuring out the whole number of chromosomes in each metaphase, as well as the chromosome number in each target class. Patau Syndrome is diagnosed if suppose there are 47 chromosomes in total and three of them are in class 13. If not, class 18's number of chromosomes will be looked at. If a person has Edwards Syndrome if they have three distinct copies of chromosome 18. Trisomy 21 is treated similarly. In contrast, Trisomy XXY, also called Klinefelter Syndrome, is found when none of the above conditions are met, and the total quantity of chromosomes is 47, and there are two chromosomes in class 23 (X class) and one chromosome in class 24 (Y class). Monosomy X occurs when class 23 has one chromosome and class 24 none. A normal case is predicted otherwise. All cases are summed up by the Table II

Aneuploidy Type	**Description**	**Number of chromosomes in Metaphase**
Normal	Autosomes 22 Pairs & Sex chromosomes 1 Pair	46
Patau Syndrome	Class 13 presented thrice	47
Down Syndrome	Class 21 presented thrice	47
Edwards Syndrome	Class 18 presented thrice	47
Klinefelter Syndrome (XXY)	Class 23 (X-chromosome) presented twice combined with one Class 24 (Y-Chromosome)	47
Turner Syndrome	Only one Class 23 Present.	45

Table II. Description of Aneuploidies

5. Discussion of Experiments and Results

5.1 Tools

Using a Personal Computer with an Intel Core(i5) @ 4.6GHz processor, NVIDIA GTX 2GBMX 230 Graphics, SSD, and 8GB RAM, we developed the suggested system using MATLAB version R2023a with Deep Learning Toolbox.

5.2 Tools

To validate our suggested method, we used the free Bioimage chromosomal categorization collection (BioImLab) images, which includes 5474 distinct grayscale chromosomal images and 117 metaphase chromosomal images in bmp file format with various sizes. The dataset was divided into portions so that training utilized 70% of the images and testing utilized 30% shown in Table III. The dataset uses data augmentation techniques because there aren't as many images overall. During training, the augmentation happens as a result of random flipping, shifting, and jittering. This dataset helps us verify the accuracy of the classification scheme we've suggested

Chromosome Types	Chromosomes Count	
	Training set	Test Set
Autosomes (1-22 types)	3036	1242
Sex chromosome (X)	398	200
Sex chromosome (Y)	398	200

Table. III. Distribution of chromosomes across classifications for the BioImLab dataset.

5.3 Experiments and Results

The proposed GLNet is used to classify individual chromosomes on metaphase images, and after a series of experiments, we discovered that the SGDM has best training parameters 10-3 learning rate, and 0.9 momentum. The model was trained for 30 epochs and 990 iterations. Each iteration has 30 epochs with validation frequency of 33 on a 5 mini batch size.

a) Measures of Performance

AP (Average precision) is the most popular and widely used metric for object detection. To calculate this metric, recall and precision values must first be determined. Recall, as defined in Equation (1), is the ratio of positively detected objects to the entire amount of objects found in a dataset.

$$Recall = \frac{TP}{TP+FN} \quad (1)$$

In which the True Positive (TP) is the opposite of the False Negative (FN).

Precision, as defined in Equation (2), is the percentage of successfully identified positively detected objects relative to the total number of successfully identified positively detected objects.

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

The term (FP) refers to False Positive.

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The average precision is computed by averaging the precision at 11 recall values equally spaced apart, as shown in Equation (3)

$$Avg\ Precision = \frac{1}{11} \sum_{Recall\ i} Precision(Recall\ i) \quad (3)$$

Precision and recall factors must be close to unity for a good classifier. The F1 score is derived by computing the harmonic mean of the two aforementioned metrics, namely Recall (R) and Precision (P). A classifier's performance is deemed superior when the F1 score is elevated.

$$F1\ score = 2 * \left(\frac{P*R}{P+R}\right) \quad (4)$$

The accuracy of classification refers to the proportion of chromosomes that have been correctly identified as belonging to the entire number of chromosomes present in the dataset. As an example,

$$\text{Accuracy} = \frac{\text{TP+TN}}{\text{TP+TN+FP+FN}} \quad (5)$$

The proposed GLNet architecture is supported by a variety of classification metrics including Recall, Precision, and F1 score, which are summarized below for each of the 23 chromosome class pairs.

Class	Recall (R)	Precision (P)	F1 Score
1	1	0.96	0.98
2	0.77	0.76	0.77
3	0.95	0.82	0.89
4	0.8	0.89	0.84
5	0.89	0.94	0.90
6	0.87	1	0.91
7	0.98	0.83	0.91
8	0.84	0.96	0.92
9	0.95	0.99	0.96
10	0.97	0.91	0.89
11	0.85	0.96	0.95
12	0.93	0.93	0.94
13	0.98	0.88	0.94
14	0.90	0.85	0.87
15	0.87	0.93	0.88
16	0.92	0.96	0.92
17	0.84	0.91	0.86
18	0.94	0.90	0.92
19	0.92	0.83	0.89
20	0.99	0.96	0.98
21	0.98	0.99	0.99
22	0.84	0.87	0.86
23	0.80	0.82	0.87
24	0.85	0.86	0.88

Table IV. Performance measures of different chromosomes classes

The accuracy of detecting abnormalities is determined by the proportion of accurately diagnosed cases in relation to the overall number of cases. Table IV summarizes all of the estimated parameters.

$$Abnormality\ detection\ accuracy = \frac{Accurately\ diagnosed\ cases}{Overall\ number\ of\ cases} \times 100 \quad (6)$$

b) Accuracy and Loss Calculations:

In the present work, the GLNet architecture was conceived from the ground up, and different learning rates were used to train the network. After much iteration with different learning rates, the starting rate was set to 0.0001 and the final rate is set to 0.0003. The augmentation of the number of epochs results in an increase in both the training and validation accuracy. The classification accuracy for SGDM optimizer was approximately 85% without any adjustments made to the learning rate. The present study involved the implementation of a learning rate upgrade strategy, which was executed after a predetermined number of epochs, specifically 30. The learning rate was held constant at 0.0003, and the SGDM optimizer was utilized. After implementing these modifications, the classification accuracy increased to approximately 96.34%. A graph was made to display the accuracy and loss figures acquired for different numbers of epochs. Figures 8 and 9 depicts the training and validation accuracy, as well as the proposed model's training and validation loss using the SGDM optimizer over a range of epochs.

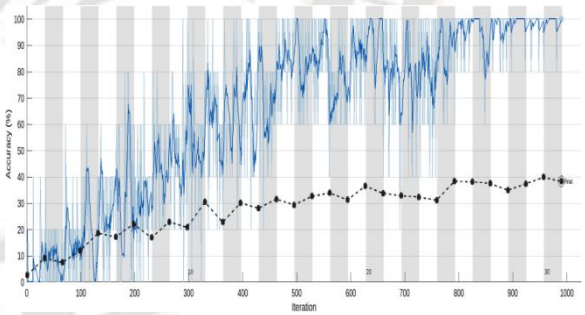

Fig. 8. Accuracy plot of the suggested model

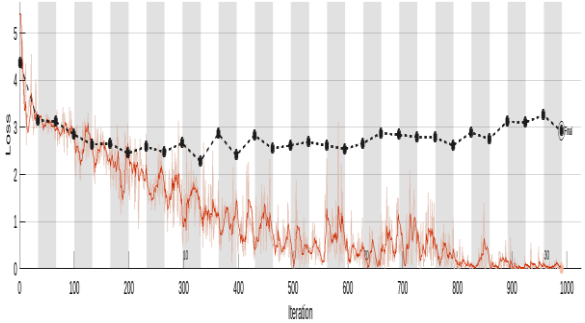

Fig. 9. Loss plot of the suggested model

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6. Conclusion

More and more cytogenetic lab techs want an automated karyotyping system that can find aneuploidies. This would help them and save them time. In our proposed work, we built from scratch a simplified CNN architecture called GLNet. It has multiple convolution layers, a GAP layer, FC layers with drop out and batch normalization, and a lot of hyperparameters that can be changed. SGDM optimizer was used in our proposed work, and the learning rate was increased every 30 epochs. Compared to other conventional optimizers, this provided improved accuracy along with lower validation loss. The utilization of various evaluation metrics, including precision, recall, support, and F1 score for distinct classes, indicates that the suggested architecture, in conjunction with the selected hyperparameters, yielded superior outcomes compared to alternative neural network-based methodologies. In the BioimLab dataset, a trained geneticist has labeled all of the training and test data that our suggested deep learning network will use. Several literary works have employed a common dataset to categorize human metaphase chromosome images. Also, the proposed method was able to classify metaphase chromosome images with an accuracy of 96.33%. This means that it can be used to diagnose aneuploidies and many other genetic disorders. We believe this survey will provide researchers with a snapshot of where the field of automatic chromosome feature extraction and neural network (NN)-based classification algorithms currently stands. This will help them come up with better ways to find abnormalities and diagnose genetic disorders

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