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A Hybrid Fuzzy Logic and Convolution Neural Network (FIS-CNN) for Automatic Detection and Classification of Objects in Comet Assay Images

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Abstract: Deep learning algorithms were able to discover many complex features in large data sets, as manually extracting features may lower the accuracy of the information in addition to wasting time, especially in huge databases, so researchers have tended to use convolutional networks to detect and classify objects in images instead of methods Former traditional. Detection of DNA damage is one of the very important topics of our time because it is responsible for diagnosing many diseases at an early date, as well as knowing the stages of disease development by determining the degree of damage to the DNA. This study is suggest a hybrid Mamdani fuzzy logic (Type-2) for detecting edges of each object of the image in (FIS-CNN) model based on preprocessing image enhancement using adaptive histogram equalization and segmenting processing in morphology operations for each object in images, then patterns of comets are detected in CNN network and classify into five scores grade automatically. The experimental results conducted on the database have achieved a high performance precision 94.34% accuracy, the propose approach compared to similar modern methods. In addition, the proposed approach is capable of detecting comets that are difficult to see with the human eye.

Keywords: Pattern recognitions, Object detection, Morphology operations, Fuzzy logic, Convolutional neural networks, Comet assay images.

1. INTRODUCTION

The modification in the DNA structure results in DNA damage. This occurs through oxidation (i.e. basic modifications modifications), as well as hard sites, and the breakdown of DNA strands [1]. The oxidative damage in DNA is an important parameter necessary to assess oxidative stress and the risk of cancer. Comet assay is a relatively simple and fast way used to assess a uni-strand/doubly-stranded DNA break. The comet assay has been used in many applications related to new chemical tests for genotoxicity, for example, the diagnosis of genetic disorders, and monitoring of genotoxins resulting from environmental pollution [2]. It is sensitive to detect low levels of DNA damage, DNA cross-links, alterations in alkaline sites, and base or base pair damages. As a method for measuring single-stranded DNA strands, it was first presented by Ostling and Johansson in 1984 [2]. In this method, membranes and soluble cell components are removed, leaving only the DNA coiled and bound to the nuclear matrix. The DNA loops resulting from alkaline treatment and electrophoresis have separators that point towards the anode, and after adding a suitable dye, the resulting ”comet tail shape can be imaged via fluorescence microscopy. The structures resulting from electrophoresis are a ”head” that reflects the super-twisting of the DNA and a ”tail” that stands for the broken and torn off the DNA. The length of the comet tail in comparison to the head stands for the extent of DNA damage [1], [2]. The correct detection of the comet score is especially critical point, as it depends mainly on researcher's experience in finding the degree of damage in DNA visually. This process is time and labor-intensive, in particular when working with many images. Therefore, automatization of this process offers the potential in reducing the time and effort spent and increase the accuracy. This comparison has been done manually [3]. It is a time-consuming and observer-dependent method. Thus, researchers have developed models to automate the detection and classification of objects in a comet assay image which is shown in Table I .

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TABLE I. COMPARISON BETWEEN STUDY STATES

Study	Method	Description
Rosati et al. [4]	R-CCN	65% accuracy for classification into 3 grades.
Erdamar et al. [5]	CNN	Classification of manually isolated objects into 5 groups
Atila et al. [6]	CNN	Classification of manually isolated objects into 4 groups
Turan et al. [7]	Dynamic time warping and decision tree	After preprocessing stage, extraction of the objects and marking of the center of the heads were done manually
Mese et al. [8]	Otsu's algorithm	Segmentation of the objects in a manually selected area.
Mani et al. [9]	a standalone tool named "CoMat" is a briefly usage of Comet Matlab	Analyze both silver stained and SYBR green stained images
Ganapathy et al. [10]	Support vector machine	Images are categorized into 2 groups (lightly/moderately damaged or heavily damaged)
Sreelatha et al. [11]	Otsu's algorithm	Minimum tail loss in Gaussian filtering was achieved by contrast enhancement in the preprocessing stage
Kızıltan et al. [12]	Radial mapping algorithm	Tail Moment was calculated as a measure of Tail Length and DNA % in the tail.
Sreelatha et al. [11]	Morphological bottom-hat transformation	Shading correction was achieved
Gyori et al. [13]	Geometric shape attributes	Detection and segmentation was done through image intensity profile analysis
Sreelatha et.al. [14]	morphological bottom-hat or top-hat transformation and Otsu's thresholding	Find highly damaged cells, then selected ROIs and hence gives better result in comet parameter quantification
Gonzalez et al. [15]	CellProfiler open-source software for automatic analysis of comets	Detection silver-stained comets and measurement DNA percentage tail
Helma et.al. [14]	Develop image-analysis system program	Automated measurement of comet-assay parameters(head,tail)
Bocker et.al. [15]	Develop automated analysis system	Automatic cell recognition and comet classification and quantification of desired comet parameters
Rivest et.al. [16]	Develop new technique to detect primary DNA damage of individual cells	Segment the comets manually by applied morphology operation and watershed transformation.

In this study, a novel method based on hybridizing fuzzy logic with CNN is proposed to increase the accuracy for all types of objects in images. Unlike traditional computing, fuzzy logic is a form of soft computing that has the advantage of absorbing inaccuracies in real world.. Soft computing enables tracking of data inaccuracies and finding solutions at a low cost. That is, fuzzy logic aims to approximate multi-valued logic rather than an optimal solution.

Unlike traditional binary logic, for example, where it represents the values of true and false variables as 1 and 0 respectively, in fuzzy logic, it represents values between 0 and 1. The membership in fuzzy logic is represented by the values 1 and 0. Means absolutely correct, 0 means completely false, while the values represented between 1 and 0 indicate the degree of correctness. That is why membership in fuzzy logic is close to the intuition of human behavior, so the applications that use fuzzy logic have been increased recently [17]. There is only one research in the published literature that did three-level detection using F RCNN As each image contains an indefinite number of objects with different level of DNA damages from (score 0 to score 4) according to the damage score, the higher the damage score, the more difficult it is to diagnose because it is more brighter, it merges with the image's successor, and it is difficult to separate it. In this paper we have several contributions that that mentioned bellow:

1) We developed a methodology that separate the objects in the images and categorized them according to their degree depending on the bibliographic classification of the objects levels according to the degree

of damage for the purpose of training set in network to get a trained network.

2) In the developed method at the testing stage the user selects a complete image that contains a number of objects, and the program automatically performs a detection and classification, each according to its score from score 0 to score4.

2. MATERIEL AND METHODOLOGY

The proposed method is an image detection method that uses the CNN for an effiecient feature extraction. The current method is intended to separate the objects in the images and categorized them according to their degree depending on the bibliographic classification of the objects levels according to the degree of damage for the purpose of training set in network to get a trained network. In Figure 1 the proposed system is clearly shown.

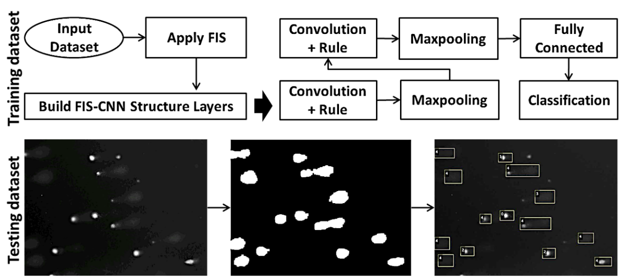

Figure 1. Proposed System (FIS-CNN) Model

A. *Mamdani Fuzzy logic for objects edge detection*

Fuzzy logic is firstly proposed by Zadeh et al. [18]. In this study, we have used the Fuzzy logic type-2 proposed by Mamdani to detect the edges [19].

The cumulative set is Fuzzy set A, the set X is universal consisting of Arranged pairs of elements of x , as in 1 [20].

$$A = \{(x, \mu_A(x)) \mid x \in X\} \tag{1}$$

where, $\mu_A(x)$ is the fuzzy function values are taken in the linearly ordered interval membership set [0,1]. Gaussian membership function , as in 2 [20]:

$$\mu_A(x) = \exp\left(\frac{(x-c)^2}{2p^2}\right) \tag{2}$$

In this context, (c) as well as (p) are considered centers and widths of a fuzzy sets where $x \in A$ The max pixels value belonging to either high or low sides of the fuzzy function, the Gauss membership function is distributed on the sets where, $x\{0, 1\}$. For detecting the edge of each object of an image, initial values of Gx=[-1,1], for crisp input values were calculated.

Fuzzification: it converts the numeric values of the input images into the equivalent membership values of the fuzzy set through membership functions, in the fuzzy set the product of the degree of membership is usually between (0,1). It used Gaussian membership functions both of as Linguistic input variables, then as output.

Defuzzification: In the degree of membership the variables are shuffled for the acceptable input images values of the fuzzy set. Then the latter extracts a specified amount of fuzzy range represented, as output of Gaussian membership functions is known triangular membership function for output results, in order to detect the correct scores of the object, otherwise incorrect detected.

B. *Processing in FIS-CNN Model*

The 10 layers created by FIS-CNN architecture are shown in Table II. CNN structure consisted of 10 layers. The images generated from the pre-processing stage enters in the first layer (input layer). Later, the convolution layers and their activation functions extract the basic features (convolution, Rectified Linear Unit (ReLU)). The maxpooling layer choses the maximum values from the upper layer. Then these features become more complex as the layers are deeper[21]. The software produces the predicted grade from the last layer (classification layer), to predict the expected outputs.

The convolution layer confirms the size of the input dataset and normalizes it [22], The CNN model includes two convolutional layers. 2D convolutional layer, as in 3 [5].

$$S\,(i, j) = (I * K)\,(i, j) = \sum_{m}^{m} \sum_{n}^{n} I\,(m, n)\,, K\,(i - m, j - n) \tag{3}$$

where, K is a kernel, S is the stride, I is the number of images, m is the width, n is the height, with the filter kernel (3×3) applied, input images size (100×100), the filter moves over the dataset images in vertical and horizontal directions. This operation is called stride (S). In order to conserve information from the edges of the images, padding (P) of the original images may be applied before the stride. Kernels can detect low level features (lines, edges, and blobs) in the first layers only, while the deeper layers are used to detect more complex features[23].

In the first convolution layer, 98×98 pixels and 10 layers images were in 10 different filters with 3×3 kernel. ReLU model equation as function of (x), where x is positive if the input and output are equal, and x is 0 for other values [24] , as in 4:

$$f\,(x) = max\,(0, x) \tag{4}$$

To reach the highest level of acceptance and to reduce the number of parameters by half in maxpooling layer (49 × 49 pixels and 10 layers, S = [2,2]) the Relu Layer is applied [22]. The second convolution layer (47 x 47 pixels and 10 layers) is scanned with convolution filters with 3 × 3 kernel. In maxpooling layer, the feature map results are reduced to 23 x 23 pixels and 10 layers.

The last advanced three layers; Fully Connected layer (FC) is used to connect each neuron in each layer. The

TABLE II. THE STRUCTURE OF THE FIS-CNN LAYERS.

Layers Num.	Layer Type	Size	Kernel Size	Stride	Padding
1	Input	100×100	-	-	-
2	Convolution_1	98×98×10	3×3	2	Same
3	Rule_1	98×98×10	-	-	-
4	Maxpooling_1	49×49×10	2×2	2	Same
5	Convolution_2	47×47×10	3×3	2	Same
6	Rule_2	47×47×10	-	-	-
7	Maxpooling_2	23×23×10	2×2	-	-
8	Full Connected	5290	-	-	-
9	Softmax	1×1×5	-	-	-
10	Classi-fication Output	5	-	-	-

classification is done at this layer. Finally, classification errors are estimated based on the entropy loss. Then the objects are labeled based on the predicted categories. The predictions were compared with manual classification. Manual classification was performed using the method proposed by Collins et al. [3].

C. Detection of Objects

1) A Contrast-Limited Adaptive Histogram Equalization (CLAHE):

was applied to the whole image to merge the small non-intersecting, and adjacent squares of each object in the image using bilinear interpolation to remove artificial borders, which has proven efficient in defining the boundaries of objects in the image [25].

2) Morphological Operation

On the binary system, morphological mathematics in 5:

$$I(I_x, I_y) \in 0, 1 \tag{5}$$

hat is applied to determine the shape of the object. Structuring Element (SE) is determined using the shape of object as in 6 [26].

$$U(U_x, U_y) \in 0, 1 \tag{6}$$

Dilation is the expansion of the object in the raster space according to the specified SE. Objects in an image will be extended depending on the SE as defined, especially in the tail part of objects over with low intensity (higher than Score-2). Therefore, threshold value = 0.1 is used, and the gaps are filled with the neighboring pixels to determine objects. The noise in the image is removed by morphology opening operation [27]. The area of labeling objects:, the centroid, and the BoundingBox of each object in the comet assay image , as in 7 [28].

$$\text{Area} = A = A_{i,j}, X \text{ ROI}\,[\text{Area}] = I, Y \text{ ROI}\,[\text{Area}] = J \tag{7}$$

In which both, i and j are pixels of shapes, ROI is the regions of interests, X ROI is a vector containing ROI x positions, Y ROI is a vector containing ROI y positions. Centroid of objects =(x′,y′), as in 8 and 9 [29]:

$$x = \frac{\sum\sum x\ f(x,y)}{\sum\sum\ f(x,y)} \tag{8}$$

$$y = \frac{\sum\sum y\ f(x,y)}{\sum\sum\ f(x,y)} \tag{9}$$

D. Performance Metric

The comet assays images were detected and identified utilizing FIS-CNN, has been measured by expert authors. The comets assays score for detection and classifications are used as the golden criteria. Thus, the FIS-CNN performance was assessed based on the obtained scores. The performance metrics of the FIS-CNN are found by calculating the accuracy precision, accuracy sensitivity, as well as the accuracy, values. The following are representing the scores:

$$Precision = TP/(TP + FP) \tag{10}$$

$$Sensitivity = TP/(TP + FN) \tag{11}$$

$$Specificity = TN/(TN + FP) \tag{12}$$

$$Acuraccy = (TP + TN)/(TP + TN + FP + FN) \tag{13}$$

In the above four equations, once can compute the precision in Equation 9 after calculating the True Positive (TP), and the False Positive (FP) [30].
As for the TP it measures the items that re truly classified to their right class while the FP are all items that are wrongly put in the wrong class.
When it comes to the sensitivity, it is calculated using Equation 10 after calculating the TP and the False Negative (FN) items [30].

3. DATASET AND RESULTS

A. DATASET: Comet assay images

The images were kindly provided by Prof. Ozlem Darcansoy Iseri (Baskent University, Turkey), and Prof. Emanuele Frontoni group (Università Politecnica delle Marche, Italy). The first process was to reduce the size of the original image from (574×768) to (100×100). In order to obtain better network reckoning performance, reduce time, simplify calculations by reducing dimensions and calculations between pixels in the image. Comet assay is sensitive technique to detect low level DNA damage in individual cells. It is a fast, cheap and reliable method. It provides important information on the early stages of DNA damage. Computer-aided diagnosis (CAD) can be used to automate the analysis of comet assay results. It is help biologist to be trust for diagnoses diseases.

B. Results

In this research, various membership values of Mamdani fuzzy logic have been used with CNN structure to detect and classify the comet assay scores. The training results are shown in Table III.

Table IV. shows the accuracy of testing results for various membership values of Mamadani Fuzzy Logic. In architecture, both padding and stride were the same. The training effectiveness of the performance matrices for precision, sensitivity, specificity, and accuracy is calculated in Table V. After training, the FIS-CNN is tested. Table VI. shows the test results of each score in three tests.

As a result, for the first test, the best precision, sensitivity, specificity, and accuracy values were obtained at 100% for score-0, while precision, specificity, and accuracy values were obtained at 100% for score-1. As for score-2 in the first test, the best precision value was obtained at 67.5%, the sensitivity value was obtained at 90%, the accuracy value was 95.15%, but the specificity value was obtained at 96.47% in the second test. For score-3 in the first test, the best precision value was 74.8%; specificity and accuracy values were obtained at 93.09% and 87.56%, respectively. However, the best sensitivity value was obtained at 78.48% in the second test. For score-4 in the second test, the best precision was obtained at 94.77%, and the specificity was obtained at 94.90%. In the first test, the best values of sensitivity were obtained at 86.6%, and the accuracy was obtained at 89.49%.The accuracy of CNN-based approach was low for these types of objects.

Implementation of the FIS-CNN approach increased not only the accuracy for low-score objects but the overall accuracy. The sample images of the implementation system are shown in Figure 3. The model begins with a preprocessing step using fuzzy logic to detect the edges of each object and increase the likelihood of detecting the objects, particularly low-score objects. The values of Gaussian membership function are equal to or close to 1. An example of deFuzzification is shown in Figure 2. First, the initial Upper membership value of 0.1 was tested, then the initial Lower membership value of 0.5 was tested, and other values were not changed.

This Mamdani Fuzzy (Type-2) in a fuzzy rule-based system step was implemented to detect edges of each object in an image. Fuzzification stage was done by converting crisp values into fuzzy values for each object in image. The fuzzy logic edge detection is shown in Figure 3.

The first test was done with an initial upper membership value of 1, then the initial lower membership value of 0.5, and other values were not changed. The second test was done with an initial Upper membership value of 0.85, then with an initial lower membership value of 0.3, and other values were not changed. The third test was done with an initial upper membership value of 0.95, then the initial lower membership value of 0.7, and other values were not changed.

In the first test, the objects in the image were correctly classified. But in the second and third tests, classification was not successful because the initial values of upper and lower memberships changed the object's score.

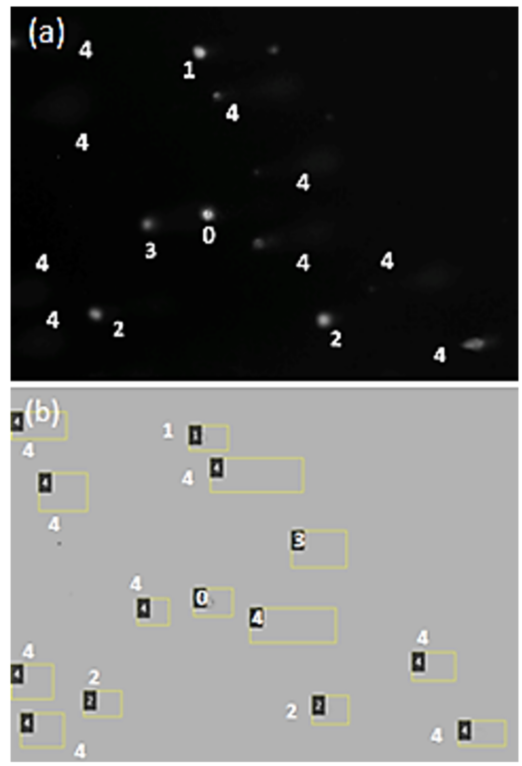

Figure 2. Sample image for edge detection(a), and object detection classification (b)

Comparing the results between our proposed study and the previous results, as shown, there is only one study that used (Faster-RCNN) to classify the objects in the image into three levels with an accuracy of 65%. As for our study, it provided a hybrid model to improve the performance of FIS-CNN detection and classification with an accuracy of 94.34% and more efficiency than base-CNN classification with an accuracy of 91.45%, as well as classify objects into five scores.

The compared results are shown in Table VII.

TABLE III. Training results of system accuracy of various membership values

Tests	First test	Second test	Third test
Membership values	[0 ,0 ,0.5] [0.1 ,1 ,1]	[0 ,0 ,0.7][0.1 ,1 ,0.95]	[0 ,0 ,0.3][0.1 ,1 ,0.85]
Accuracy	99.40%	96.65%	91.95%

TABLE IV. Testing results of system accuracy of various membership values

Tests	First test	Second test	Third test
Membership values	[0 ,0 ,0.5] [0.1 ,1 ,1]	[0 ,0 ,0.7][0.1 ,1 ,0.95]	[0 ,0 ,0.3][0.1 ,1 ,0.85]
Accuracy	94.34%	90.52%	88.66%

TABLE V. Training results of system accuracy of various membership values

Scores	First test	Second test	Third test
score-0	100%	98.90%	97.86%
score-1	100%	97.55%	96.33%
score-2	99.15%	96.22%	94.11%
score-3	98.39%	95.48%	93.89%
score-4	99.46%	95.12%	92.23%
Total	99.40%	96.65%	94.88%

TABLE VI. Testing results of system overall tests

	Scores	Precision	Sensitivity	Specificity	Accuracy
First test	score-0	100%	100%	100%	100%
	score-1	100%	91.6%	100%	99.51%
	score-2	67.5%	90%	95.70%	95.15%
	score-3	74.8%	77.2%	91.10%	87.56%
	score-4	94%	86.6%	93.09%	89.49%
	Total	87.26%	89.08%	95.97%	94.34%
Second test	score-0	65%	61.90%	98.82%	97.57%
	score-1	52.27%	63.88%	96.39%	94.50%
	score-2	55.17%	80%	96.479%	91.76%
	score-3	69.27%	78.48%	88.06%	85.62%
	score-4	94.77%	73.83%	94.90%	83.19%
	Total	67.20%	71.61%	94.93%	90.52%
Third test	score-0	66.66%	47.61%	98.99%	97.41%
	score-1	41.86%	50%	95.71%	93.05%
	score-2	53.33%	56.66%	93.73%	91.11%
	score-3	47.40%	65.82%	75.27%	72.85%
	score-4	91.07%	59.30%	92.72%	88.89%
	Total	60.06%	55.87%	91.28%	88.66%

TABLE VII. comparing results among three models.

Model	Grade/Accuracy					
Faster-RCNN [4]	Low 70%	Medium 51%	High 74%			Total 65%
CNN (previous study)	Score-0	Score-1	Score-2	Score-3	Score-4	Total
	94.34%	93.33%	93.86%	88.52%	87.23%	91.45%
FIS-CNN (proposed)	00%	99.51%	95.15%	87.56%	89.49%	94.34%

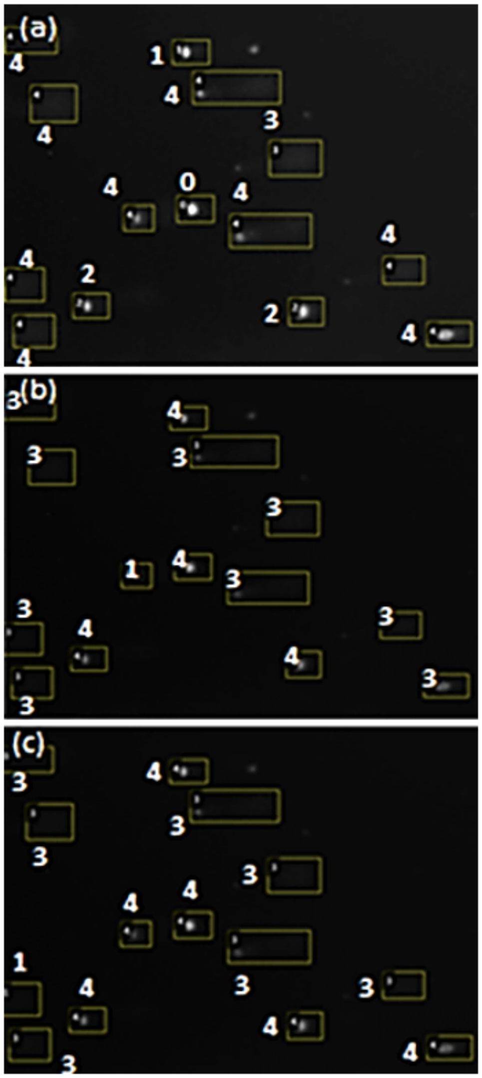

Figure 3. Correct score of object detection with upper membership value 1 and lower membership value 0.5(a), Incorrect score of object detection with upper membership value 0.85 and lower membership value 0.3(b), and Incorrect score of object detection with upper membership value 0.95 and lower membership value 0.7 (c)

4. DISCUSSION

The quantitative analysis was done manually by specialized experts for the comet assay images. Various software metrics were used to calculate the degree of DNA damage. In manual evaluation, the scores of DNA damage from (score-0 to score-4) are expressed from least to most progressive. Correct detection of the score of DNA damage is very important in order to determine the score of DNA damage. Scoring large numbers of images is exhausting and consumes time.

The approach used in previous studies to detect DNA damage focused on the features of different spatial parameters extracted from the images, and the images were classified using these parameters. Such methods negatively affect the characteristics of the images. For example, images from grey staining are not the same as those from fluorescent staining because the former is noisier than the latter, resulting in a classification failure to detect the degree of comet damage using systematic methods. Also, fluorescent staining images have variable properties in brightness and contrast. This difference can be attributed to differences in experimental numbers and conditional differences in laboratories. It is one of the possible reasons that led to these differences. In this study, a new approach to computer-aided to automatically detect and classify comet scores using a hybrid FIS-CNN was used to show five scores with different patterns. The results were robust for detecting and classifying comets in different scores of the comet assay.

5. CONCLUSION

Hybrid FIS-CNN model was developed to detect objects in comet assay images and classify them based on the extent of DNA damage. The total accuracy was 94.34%, for score-2 and lower objects was over 95%. This model is expected to increase the accuracy and reduce the time needed for comet assay analysis compared to manual analysis method. To the best knowledge of the authors, this is the first method for automatic detection and classification of images with multiple objects using the standard classification of comet assay shows good performance results for determining objects scoring classification and successful implementation for a sensitive images in medical images like comet assay images. To improve performance accuracy of (FIS-CNN) model by acquiring more comet assay images to increase the dataset, currently 261 images has been used, we aim to get 1000 images.

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References

[1] C. P. Gonzalez-Hunt, M. Wadhwa, and L. H. Sanders, "Dna damage by oxidative stress: Measurement strategies for two genomes," *Current Opinion in Toxicology*, vol. 7, pp. 87–94, 2018.

[2] O. Ostling and K. J. Johanson, "Microelectrophoretic study of radiation-induced dna damages in individual mammalian cells," *Biochemical and biophysical research communications*, vol. 123, no. 1, pp. 291–298, 1984.

[3] A. R. Collins, "Investigating oxidative dna damage and its repair using the comet assay," *Mutation Research/Reviews in Mutation Research*, vol. 681, no. 1, pp. 24–32, 2009.

[4] R. Rosati, L. Romeo, S. Silvestri, F. Marcheggiani, L. Tiano, and E. Frontoni, "Faster r-cnn approach for detection and quantification of dna damage in comet assay images," *Computers in Biology and Medicine*, vol. 123, p. 103912, 2020.

[5] A. Erdamar and M. F. Aksahin, "Multi-scale classification of single-cell gel electrophoresis assay using deep learning algorithm," *Biomedical Signal Processing and Control*, vol. 56, p. 101672, 2020.

[6] Ü. Atila, Y. Y. Baydilli, E. Sehirli, and M. K. Turan, "Classification of dna damages on segmented comet assay images using convolutional neural network," *Computer Methods and Programs in Biomedicine*, vol. 186, p. 105192, 2020.

[7] M. K. Turan and E. Sehirli, "A novel method to identify and grade dna damage on comet images," *Computer methods and programs in biomedicine*, vol. 147, pp. 19–27, 2017.

[8] A. K. Meşe, A. Erdamar, and Ö. D. İşerı, "Image analysis for single cell gel electrophoresis," in *2017 25th Signal Processing and Communications Applications Conference (SIU)*. IEEE, 2017, pp. 1–4.

[9] U. Mani and P. Manickam, "Comat: An integrated tool for comet assay image analysis," *Journal of Pharmaceutical Sciences and Research*, vol. 9, no. 6, p. 919, 2017.

[10] S. Ganapathy, A. Muraleedharan, P. S. Sathidevi, P. Chand, and R. P. Rajkumar, "Cometq: An automated tool for the detection and quantification of dna damage using comet assay image analysis," *computer methods and programs in biomedicine*, vol. 133, pp. 143–154, 2016.

[11] G. Sreelatha, A. Muraleedharan, P. Chand, R. P. Rajkumar, and P. Sathidevi, "An improved automatic detection of true comets for dna damage analysis," *Procedia Computer Science*, vol. 46, pp. 135–142, 2015.

[12] E. Kiziltan and E. Yurtcu, "Semi-automatic scoring tool for comet assay," in *2015 IEEE 15th International Conference on Bioinformatics and Bioengineering (BIBE)*. IEEE, 2015, pp. 1–3.

[13] J. González, I. Romero, J. Barquinero, and O. García, "Automatic analysis of silver-stained comets by cellprofiler software," *Mutation Research/Genetic Toxicology and Environmental Mutagenesis*, vol. 748, no. 1-2, pp. 60–64, 2012.

[14] C. Helma and M. Uhl, "A public domain image-analysis program for the single-cell gel-electrophoresis (comet) assay," *Mutation Research/Genetic Toxicology and Environmental Mutagenesis*, vol. 466, no. 1, pp. 9–15, 2000.

[15] W. Böcker, W. Rolf, T. Bauch, W.-U. Müller, and C. Streffer, "Automated comet assay analysis," *Cytometry: The Journal of the International Society for Analytical Cytology*, vol. 35, no. 2, pp. 134–144, 1999.

[16] J.-F. Rivest, M. Tang, J. McLean, and F. Johnson, "Automated measurements of tails in the single cell gel electrophoresis assay," in *Quality Measurement: The Indispensable Bridge between Theory and Reality (No Measurements? No Science! Joint Conference-1996: IEEE Instrumentation and Measurement Technology Conference and IMEKO Tec*, vol. 1. IEEE, 1996, pp. 111–114.

[17] D. E. Nirmala and V. Vaidehi, "Comparison of pixel-level and feature level image fusion methods," in *2015 2nd international conference on computing for sustainable global development (INDIACom)*. IEEE, 2015, pp. 743–748.

[18] L. A. Zadeh, "The concept of a linguistic variable and its application to approximate reasoning—i," *Information sciences*, vol. 8, no. 3, pp. 199–249, 1975.

[19] E. H. Mamdani and S. Assilian, "An experiment in linguistic synthesis with a fuzzy logic controller," *International journal of man-machine studies*, vol. 7, no. 1, pp. 1–13, 1975.

[20] F. Orujov, R. Maskeliūnas, R. Damaševičius, and W. Wei, "Fuzzy based image edge detection algorithm for blood vessel detection in retinal images," *Applied Soft Computing*, vol. 94, p. 106452, 2020.

[21] M. Habibzadeh, M. Jannesari, Z. Rezaei, H. Baharvand, and M. Totonchi, "Automatic white blood cell classification using pre-trained deep learning models: Resnet and inception," in *Tenth international conference on machine vision (ICMV 2017)*, vol. 10696. SPIE, 2018, pp. 274–281.

[22] A. Krizhevsky, I. Sutskever, and G. E. Hinton, "Imagenet classification with deep convolutional neural networks," *Communications of the ACM*, vol. 60, no. 6, pp. 84–90, 2017.

[23] Y. LeCun, L. Bottou, Y. Bengio, and P. Haffner, "Gradient-based learning applied to document recognition," *Proceedings of the IEEE*, vol. 86, no. 11, pp. 2278–2324, 1998.

[24] V. Nair and G. E. Hinton, "Rectified linear units improve restricted boltzmann machines," in *Icml*, 2010.

[25] K. Zuiderveld, "Contrast limited adaptive histogram equalization," *Graphics gems*, pp. 474–485, 1994.

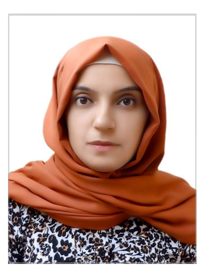
[26] J. Balado, P. Van Oosterom, L. Díaz-Vilarino, and M. Meijers, "Mathematical morphology directly applied to point cloud data," *ISPRS Journal of Photogrammetry and Remote Sensing*, vol. 168, pp. 208–220, 2020.

[27] L. Vincent, "Morphological area openings and closings for greyscale images," in *Shape in Picture*. Springer, 1994, pp. 197–208.

[28] K. Suzuki, J. Shiraishi, H. Abe, H. MacMahon, and K. Doi, "False-positive reduction in computer-aided diagnostic scheme for detecting nodules in chest radiographs by means of massive training artificial neural network1," *Academic Radiology*, vol. 12, no. 2, pp. 191–201, 2005.

[29] C. Fosu, G. Hein, and B. Eissfeller, "Determination of centroid of ccd star images," *Int. Arch. Photogram. Remote Sens. Spatial Inform. Sci*, vol. 35, pp. 612–617, 2004.

[30] T. Fawcett, "An introduction to roc analysis," *Pattern recognition letters*, vol. 27, no. 8, pp. 861–874, 2006.



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