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THE AUTOMAIC DETECTION OF RNA VIRUSES USING MULTI-ENTROPY AND ARTIFICIAL NEURAL NETWORK METHOD (ME-ANN)

ABSTRACT

Nowadays, there are many studies on microbiologic diagnosis literature. In this study, the Multi-entropy and Artificial Neural Network (ME-ANN) system is presented for automatic detection of RNA virus images. This system consists of four stages. They are respectively pre-processing, feature extraction, classification and test of correct detection ratio of this ME-ANN method. In pre-processing stage, it is used the center - edge changing method. In this method, Euclidian distances are calculated the from center pixels of an object on image to edges of this object. Therefore, the distance vector has been obtained. This calculating is repeated for each of RNA virus images used in this study. In feature extraction stage, the norm, the logarithmic energy and threshold entropy values are calculated as feature vector. The obtained these features are invariant from rotation and scale of these RNA virus images. In classification stage, these obtained feature vector is given to the ANN classifier. Finally the test stage is performed for evaluation the correct detection ratio of ME-ANN algorithm for RNA virus images. The correct detection ratio of the proposed system is 94.02%.

Keywords: RNA Virus Images, Center-Edge Changing Method, Entropy, ANN Classifier, Invariant Features From Scaling and Rotating.

ÇOKLU ENTROPİ VE YAPAY SİNİR AĞI YÖNTEMİ (ÇE-YSA) KULLANARAK RNA VİRÜSLERİNİN OTOMATİK BULUNMASI

ÖZET

Son zamanlarda, mikrobiyoloji teşhis literatürü üzerine birçok çalışma yapılmıştır. Bu çalışmada RNA virüs görüntüsünün otomatik bulunması için çoklu entropi ve yapay sinir ağı (ÇE-YSA) sistemi sunulmuştur. Bu sistem dört adımdan oluşmaktadır. Bunlar sırasıyla ön işleme, özellik çıkarma, sınıflandırma ve ÇE-YSA yöntemin doğruluğunun başarısının test edilmesidir. Ön işleme adımında merkez kenar değişim yöntemi kullanılmıştır. Bu yöntemde görüntü üzerindeki bir nesnenin merkez piksellerinden nesnenin kenarına olan Euclidean mesafesi hesaplanmıştır. Bu yüzden mesafe vektörü elde edilmiştir. Bu hesaplamalar çalışmada kullanılan RNA virüs görüntülerinin her biri için tekrar edilmiştir. Özellik çıkarma adımında özellik vektöründen norm, logaritmik entropi ve eşik entropi değerleri hesaplanmıştır. Elde edilen bu özellikler RNA virüs görüntülerinin döndürülmesi ve ölçeklenmesinde değişmezdir. Sınıflandırma adımında elde edilen özellik vektörleri YSA sınıflandırıcıya verilir. Son aşamada RNA virüsleri için ÇE-YSA algoritmasının doğruluğunun başarı oranının test edilmesi gerçekleştirilmiştir. Önerilen sistemin başarı oranı %94.02'dir.

Anahtar Kelimeler: RNA Virüs Görüntüleri, Merkez-Kenar Değişim Yöntemi, Entropi, YSA Sınıflandırma, Ölçekleme ve Döndürmede Değişmez Özellikler

1. INTRODUCTION (GİRİŞ)

Viruses are microscopic particles, which are capable of infecting living cells. But a host cell infected by viruses can replicate. The most basic form of a virus is a protein called campsite genetic material consists of covers within [1-9]. Only plant and animal cells such as bacteria and organisms can multiply with the help of virus [2-9]. The material of inheritance in viruses can be DNA or RNA. This material of inheritance is called as genom.
The viruses, which carry RNA molecules as administrator, multiply by using only the cells of living organisms such as DNA viruses. Process of cell multiplication of RNA viruses occurs in cytoplasm.

The viruses can be separated according to their lives and the disease group and nucleic acids, which are carried by them. Virus RNA molecules are executives. Some animal cells and plant cells living viruses are RNA viruses. For example, tobacco mosaic virus, influenza, polio, measles, rabies, mumps, yellow fever causing viruses are RNA viruses. Replication stage of the RNA virus and some of the characteristics of viruses vary and are divided into four groups.

Double yarn RNA viruses: The virus outside the host cell, while a free particle, is called the virion. Each double-stranded RNA segment patterns as negative strand RNA molecules using single-strand synthesis is positive. These RNAs and mRNA do the task, as well as molds for the new function will see. The RNA will be produced. Thus, new double-stranded RNAs new generation need to be synthesized [2-4].

RNA genome with positive polarity of single yarn containing viruses: RNA polymerase in the virion is enzyme. Parental RNA serves as mRNA. The virus does not enter into the cell protein synthesis that makes the first ever viral RNA polyribosome. This is a fairly large protein molecule and proteins to create functional proteases with small pieces should be divided. During the positive strand replication on the new negative strands are synthesized [2-4].

RNA genomes with negative polarity of single yarn containing viruses: Each segment with a negative polarity is located inside the virion RNA positive by polymerase enzymes. Same negative pattern can be more than one positive strand synthesis. This mRNA serves as a positive RNAs, as well as for the synthesis of new negative strands of RNA serves patterns [2-4].

Reverse transcriptase containing viruses: These viruses contain single-stranded RNA positive. In addition, virion reverse transcriptase and endonuclease enzymes are RNAs-H Reverse transcriptase enzyme, which means reverse transcription. Reverse transcriptase enzyme synthesizes DNA by using RNA as form [2-4].

Found in the literature that some types of RNA viruses can be summarized as follows:

- **Picornaviruses:** These viruses are member of the Picornaviridia family. Picornaviruses have 25-30 nm size, single-stranded, positive polarity RNA, enveloped, virus icosahedral protein are sheathed. Enterovirus in this family, Hepatovirus, Rhinovirus families are lower. This family of viruses that cause diseases of aseptic meningitis, paralytic poliomyelitis, aseptic meningitis, myopericarditis, aseptic meningitis, RAS, acute hepatitis, cold.
- **Togaviruses:** These viruses are member of the Togaviridae family. Togaviruses have 60-70 nm size, single-stranded, positive polarity RNA, enveloped, virus icosahedral protein are sheathed. This family includes the Alphavirus and Rubivirus subfamily. This family of viruses that cause diseases mosquito source encephalitis, rubella, mild rash, congenital fetal defects.

- **Flaviviruses:** These viruses are member of the Flaviviridae family. Flaviviruses have 45-55 nm size, single-stranded, positive polarity RNA, enveloped, icosahedral protein sheath, the virus that is replicated in the cytoplasm. This family includes the Flavivirus and Hepacivirus sub-families. This family of viruses that cause diseases mosquito-source fever, hepatitis, yellow fever, mosquito-source hemorrhagic fever, Dengue fever, encephalitis and hepatitis C (acute/chronic), liver cancer.
- **Orthomyxoviruses:** These viruses are member of the Orthomyxoviridae family. Orthomyxoviruses have 80-120 nm size, 8-piece, single-stranded negative polarity RNA, enveloped, helical protein are sheathed. Subfamily of this family are called Influenzae virus. This family of viruses that cause influenza disease, fever, myalgia, fatigue, cough, pneumonia.
- **Bunyaviruses:** These viruses are member of the Bunyaviridae family. Bunyaviruses have 100 nm size, enveloped, helical protein are sheathed. Hantavirus of this family, subfamily are Bunyavirus and Phlebovirus. This family of viruses that cause pulmonary diseases originated rodent diseases, hemorrhagic fever with renal syndrome (HUS), mosquito-source encephalitis, hemorrhagic fever.
- **Astroviruses:** These viruses are member of the Astroviridae family. Astroviruses have 110-130 nm size, enveloped, helical protein sheath. Bergel ribosomes is located in the viral envelope. Human viruses of this family are most important to people astrovirus. This family of viruses that cause diseases gastroenteritis.
- **Retroviruses:** These viruses are member of the Retroviridae family. Retroviruses have 90 to 120 nm in diameter, single-stranded RNA or have complex built, are enveloped viruses. Oncovirinae in this family and the Lentivirinae subfamily includes Spumavirinae. These families are most important to the human viruses Human T Lenfotropik viruses (HTLV) and which factors AIDS Human Immunodeficiency Virus (HIV) is located. The most important feature of this virus in RNA-dependent DNA polymerase that is the existence of reverse transcriptase enzyme.
- **Coronavirus:** Coronavirus has approximately 120nm size. It is single-stranded, positive polarity RNA (mRNA) containing. Reach lengths of 20 nm length on the envelope that contains peplomers. This view gained the crown of the virus allows peplomers. This family of viruses that cause diseases like colds are mild infections [4].
- **Reoviruses:** This virus has a diameter of 60-80 nm. The center has a common two-layer kapsit. (Rotaviruses have three-layer structure.) Rotaviruses are 132 capsomery envelope. The full infectious virus is a form of double-layered particles. Reoviruslar are double-stranded, partial RNA viruses containing medium. 3.5 million many preschool children per year in developing countries are estimated to cause death. Acute gastroenteritis in the United State is domestic disease as a cause of acute respiratory infections [5-10].
- **Rhabdoviruses:** Rhabdoviridae family has 180x75 nm size. These families are families in lower Vesiculovirus and Lyssavirus. The most important peoples are Rabiesvirus viruses, Marburg virus and Ebolavirus to this family. This family of viruses that cause rabies and hemorrhagic fever diseases [11].

- **Filaviruses:** Filaviridae family has 80 nm sizes. Helical protein is sheathed. These families are most important to the human viruses. This family of viruses that cause hemorrhagic fever disease.
- **Paramyxoviruses:** Paramyxoviridae family has 150 nm size. RNA structures are single-stranded negative polarity. In this family, Paramyxovirus, Morbillivirus and Pneumovirus subfamily are available. Viruses of this family are most important to human parainfluenza virus type 1, 2, 3, 4, 5, Mumps (Mumps) virus, Measles (measles) virus. This family of viruses that cause colds diseases, bronchiolitis, pneumonia (4 types), mumps, aseptic meningitis (rarely orchitis and encephalitis), measles, fever, rash, pneumonia (rarely, encephalitis, SSPE) and cold (adult), bronchiolitis, pneumonia (YD).

2. RESEARCH SIGNIFICANCE (ÇALIŞMANIN ÖNEMİ)

So far, the virus RNA detection, these viruses are produced and in cell culture, using electron microscopy, the eyes are made with the decision. In this case, the error rate of specialists is increasing. This is to minimize the error rate for the purpose of this study, the images obtained RNA viruses and Artificial Neural Network Multi-entropy (ME-ANN)-based automatic detection system has been tried to be realized. This automatic virus RNA detection system, consists of four parts as structural. They are respectively pre-processing, feature extraction, classification and are being tested. In pre-processing stage, it is used the center - edge changing method. In this method, Euclidian distances are calculated the from center pixels of an object on image to edges of this object. Therefore, the distance vector has been obtained. This calculating is repeated for each of RNA virus images used in this study. In feature extraction stage, the norm, the logarithmic energy and threshold entropy values are calculated as feature vector. The obtained these features are invariant from rotation and scale of these RNA virus images. In classification stage, these obtained feature vector is given to the ANN classifier. Finally the test stage is performed for evaluation the correct detection ratio of ME-ANN algorithm for RNA virus images. The correct detection ratio of the proposed system is 94.02%.

3. DIGITAL IMAGE PROCESSING STAGES (SAYISAL GÖRÜNTÜ İŞLEME ADIMLARI)

In literature, there are very large digital image processing hardware and software. In Fig. 1, the basic steps on image processing are given [8-13].

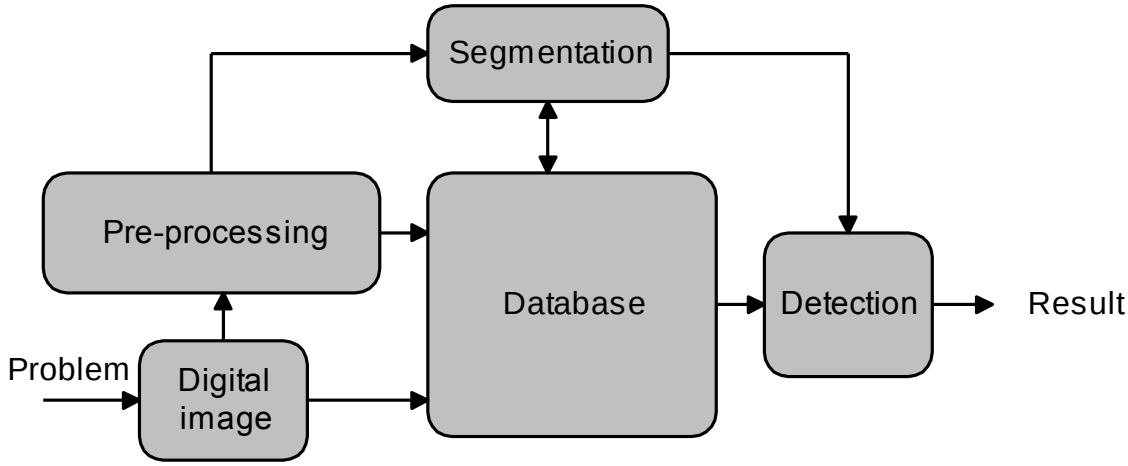


Fig. 1. The basic steps on image processing
(Şekil 1. Temel görüntü işleme adımları)

The first step is to obtain digital image in image processing. To do this, a sensor and sensor output must be digitized in the mark. The image sensor can be determined according to the application. Digital image obtained after the pre-stage process is the next step. In pre-production stage of the process followed to obtain better results in image. This pre-production stage contains the contrast, expansion, noise elimination process. Partitioning operation is performed in the third stage. Segmentation is to dissect image within its structure. Autonomous segmentation of digital image processing is one of the most difficult process. After segmentation, the output is the raw data. This data must be making the computer can process. At this point, the decision must be given to the outer limits of the data in the image of an object belonging to whether you get the inside line [12-14].

This section also called pre-processing and feature extraction stage. At this stage, the basic morphological operations are introduced with the following order.

- **Gray Level Screening:** Color images on the computer to process the image are converted to black and white images of 0 and 1s. Here, 0s, 1s represents the black and white color image respectively.
- **Histogram Indication:** Histogram indication is used to show the gray level distribution of image.
- **Image Thresholding:** Thresholding is one of the most important approach for purposes of image segmentation [10]. At thresholding objective process, the image objects within the image is separated from the background.

The distribution of gray level image (image histogram) is used for thresholding image. According to this histogram, the objects and background pixels to belong to the image can be evaluated in two main groups. In this case, the easiest way to distinguish the object from the background is histogram. The threshold value T is determined by using histogram. A threshold value T will be to compare the pixel values in image. Accordingly, for any (i, j), if the f (i, j) pixel is bigger than T if (i, j) pixel, this f (i, j) pixel belongs to a point object, else this f (i, j) pixel belongs to the plan will be a point in the image.

- **Canny Edge Extraction Method:** Edge extraction is one of the basic important issue of image processing. Edges in an image

change itself against drops brightness, color, texture, and shows. Canny edge extraction method is a multi-step extraction technique for an edge.

The main purpose of this method is a good image to the edges of objects unearthed. Canny edge extraction algorithm contains items are listed in below:

Unwanted details in the structure are reduced by passed (m, n) size image f (m, n) through a Gaussian filter noise. This process is also expressed at Eq.(1) in below:

$$g(m,n) = G_{\sigma}(m,n).f(m,n) \quad (1)$$

Here, $G_{\sigma} = \frac{1}{\sqrt{2\pi\sigma^2}} \exp\left(-\frac{m^2+n^2}{2\sigma^2}\right)$ is in the form of a Gaussian filter functions.

- **The Center-Edge Changing Method:** The center - edge changing method is one-dimensional of a two-dimensional representation of the border. The center - edge changing method can be defined as a drawing. r in the function is shape and this shape of the center of gravity is located on the border between any point represents the Euclidian distance. This calculating of Euclidian distance process is also expressed at Eq.(2) in below [13-17]:

$$r = \sqrt{(x - x_m)^2 + (y - y_m)^2} \quad (2)$$

Where x is the horizontal component of the border on the point, xm is the horizontal center of gravity point, y is the vertical component of the border on the point, ym is the vertical center of gravity point. Fig. 2 is at any point on the boundary. In addition to this point, the horizontal position represents the reference angle. The graphic in fig.2 shows the sequence of Euclidian distance, which is between center point of square and each points of edges of square [12].

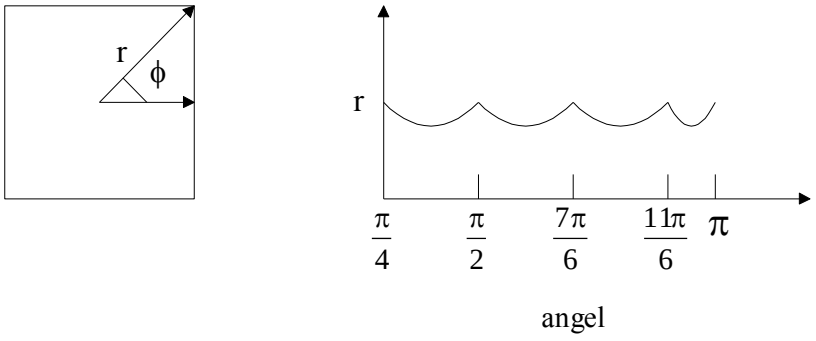


Figure 2. Application of the center - edge change method for square.
(Şekil 2. Kare şekli için merkez-kenar değişimi yönteminin uygulaması)

- **Concept of Entropy:** The concept of entropy has been first used in signal processing and especially in the field of communication by Shannon [13-16]. Entropy-based features give information about signal definitions. The concept of entropy may be a system with the aim of measuring the regularity of thermodynamics. It is a concept well known in physics. Entropy measurement method is to measure the degree of the disorder of any sign [14].

In recent years, the entropy is widely used in the field of signal processing has become a concept. The equations of type of

entropy widely used in signal processing are given below [3-7]:

Shannon Entropy:

$$E(s) = -\sum_i s_i^2 . \log_2(s_i^2) \quad and \quad \log(0) = 0 \tag{3}$$

Norm Entropy:

$$E(s) = \sum_i |s_i|^p \quad and \quad 1 \le p < 2 \tag{4}$$

Logarithmic Energy Entropy:

$$E(s) = \sum_i \log_2(s_i^2) \quad and \quad \log(0) = 0 \tag{5}$$

Entropy Threshold:

$$E(s) = \sum_i E(s_i) \tag{6}$$

ε threshold is a positive

$$|s_i| > \varepsilon \Rightarrow E(s_i) = 1 \ \ and \ \ |s_i| \le \varepsilon \Rightarrow E(s_i) = 0$$

Sure Entropy:

$$|s_i| \le \varepsilon \Rightarrow E(s) = \sum_i \min(s_i^2, \varepsilon^2) \tag{7}$$

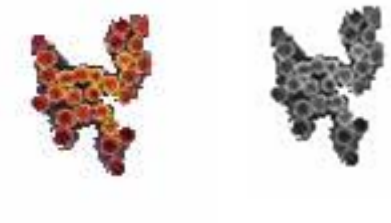
ε is a positive threshold in where.

4. USED METHODS AND STAGES (YÖNTEM VE ADIMLAR)

The RNA virus images are considered in this study were taken from the web site of the University of Pompeu Fabra, division of biology [2]. These digital images can be used for educational and non-commercial without any permission as it was noted in the related web site [2]. A total of 12 typical RNA virus images were chosen as follows: Picornaviruses (PV), Togaviruses (TV), Flaviviruses (FV), Orthomyxoviruses (OV), Bunyaviruses (BV), Astroviruses (AV), Retroviruses (RV), Coronaviruses (CV), Reoviruses (REV), Rhabdoviruses (RHV), Filoviruses (FLV), Paramyxoviruses (PRV). This application is mainly pre-processing, feature extraction, classification and testing phases of four parts to have occurred. During these stages are given below with:

- **Stage (Pre-Processing Stage):** In this stage, the pre-processing steps described in Chapter 3 are used. The above-mentioned RNA viruses in first part of this stage have been translated into images from color to gray. The images are converted into gray level image. Then, gray-level image histograms of these gray level RNA virus images are obtained. Threshold value is determined by utilizing the image histogram for each of these RNA virus images. This value is determined according to the value above the threshold value for the pixels in the image output value in the bottom 1 has been assigned a value of 0. Thus, the picture is separated from the background. Separated from the background image that we apply the Canny edge extraction algorithm, the edges of objects in these images were determined. Finally, the center-edge changing method is applied to these RNA virus images.
- **Morphological and Logical Operations:** These are the structural processes. Sometimes, an image of the object and the background to distinguish from each other to determine the appropriate threshold values may not be sufficient. In this case, we need to do additional processing. The object can be separated from the

background. That these techniques are performed using some morphological and logical operators. The morphological and logical operators used in this study are gray gevel screening, histogram indication, image thresholding, Canny edge extraction method, the center - edge changing method respectively. Application results of these morphological and logical operations for RNA viruses can be given in Figs.3-5.



(a) (b)

Figure 3. RNA virus picture, a) Astrovirus type of RNA virus, b) the gray image was converted into a state of the virus
(Şekil 3. RNA virüs imgesi, a)Astrovirus RNA virüsü, b)virüs imgesinin gri seviyeye dönüştürülmüş hali)

(a) (b)

Figure 4. RNA virus picture,
a) Astrovirus type of RNA virus, b) is the image segment
(Şekil 4. RNA virüs imgesi, a)Astrovirus RNA virüsü, b)bölütlenmiş imge)

(a) (b)

Figure 5. RNA virus Picture, a) Astrovirus type of RNA virus, b) Canny edge detection process after the image
(Şekil 5. RNA virüs imgesi, a)Astrovirus RNA virüsü, b) Canny kenar çıkarma yöntemi sonunda elde edilen imge)

- **Stage (Feature Extraction):** At this stage, the RNA virus images pre-processed in 1. Stage (Pre-Processing Stage) are used. In this feature extraction stage, each of these RNA virus images are rotated the between 0° and 165° with an angle of 15 degrees. Therefore, 12 rotated images with different angles are obtained from each of these RNA virus images.

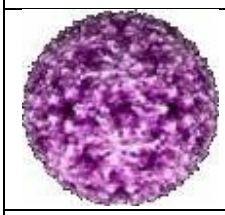
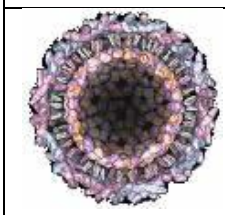
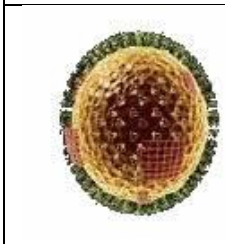
Then, obtained these rotated RNA virus images are scaled in 10 different sizes. Therefore, number of obtained total RNA virus images is 12 x 12 x 10 = 1440. Moreover, three different entropy values, which are norm, the logarithmic energy and entropy threshold values, are calculated for each of these 1440 RNA virus images. Thus, the size

of obtained feature vector at final of feature extraction stage is 1440 x 3.

The half of this 1440 x 3 size feature vector is used for classification stage. Namely, 720 x 3 (12 x 60 x 3) feature vector is given to the inputs of ANN classifier. The rest of this 1440 x 3 size feature vector is used in stage of testing of correct classification performance of ME-ANN method used in this study.

The some of the calculated entropy values of RNA viruses image can be given in Table 4:

Table 4. Some of the calculated entropy values of RNA viruses image (Tablo 4.RNA virüs imgelerinin hesaplanmış entropi değerlerinden bazıları)

Before pre-processing	After pre-processing	E1: The value of norm entropy	E2: The value of logaritmic energy entropy	E3: The value of threshold entropy
		562.0537	1.5994e+004	1595
Picornavirus				
		716.2803	1.8434e+004	1724
Togavirus				
		456.9634	1.2865e+004	1275
Flavivirus				
		478.1944	1.3618e+004	1340
Orthomyxovirus				

- **Stage (The Realized Classification by using Artificial Neural Network Classifier):** This stage is the classification stage. Here, the half of 1440 x 3 (12 x 120 x 3) feature vector obtained in feature extraction stage is used for classification. This feature vector is given to inputs of ANN classifier. The

rest of this 1440 x 3 (12 x 120 x 3) feature vector obtained in feature extraction stage is used for testing the correct detection ratio of ME-ANN algorithm for RNA virus images in testing stage. The training parameters of multi-layer artificial neural network classifier used in this study can be given on Table 5.

Table 5. Multi-layer neural network structure and training parameters.
(Tablo 5.Çok katmanlı yapay sinir ağları yapısı ve eğitim parameterleri)

Number of layers	3
The Number of Layer Nerons	Input: 3, Hidden Layer: 20, Output: 12
Initiate weights and biases	The Nguyen-Widrow method
Aktivation functions	Log-sigmoid
Training parameters Learning rule	Back propogation
Mean square error	0.00001

These values, for example, the number of hidden layers, the number of cells in hidden layers, learning rate and the value of the activation function, after several tries, could have been selected for the best performance. Multi-Entropy and Artificial Neural Networks (ME-ANN) method is developed for automatic classification of types of RNA viruses from RNA virus images. The structure of this ME-ANN algorithm is given in Fig. 6.

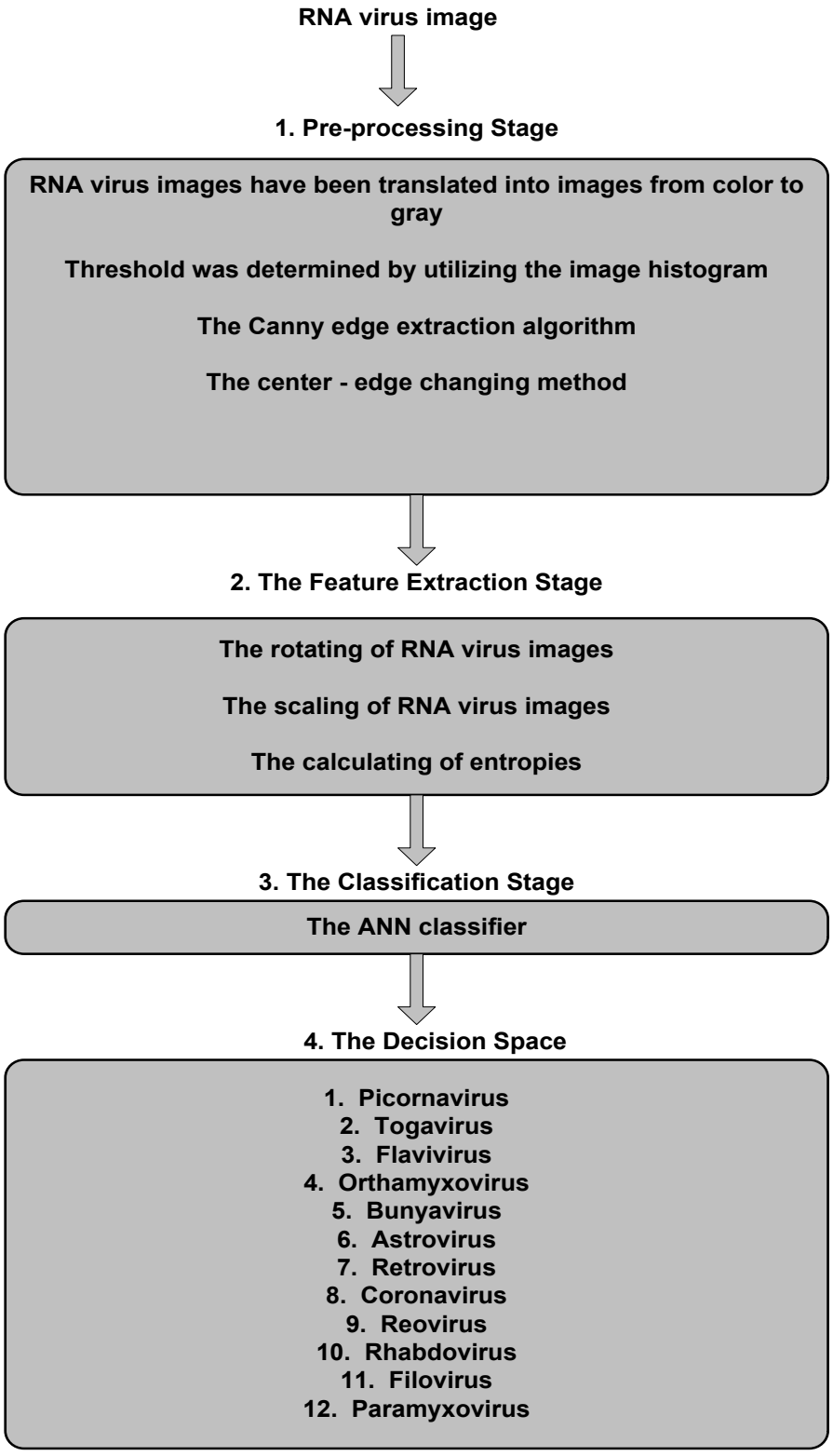


Figure 6. The structure of ME-ANN algorithm is used in this study.
(Şekil 6. Çalışmada kullanılan ÇE-YSA algoritmasının yapısı)

- **Stage (Testing of Correct Classification Performance):** This is the 4th stage is accomplished by using ME-ANN algorithm is testing the accuracy of classification results. For this purpose, the rest of this 1440 x 3 (12 x 120 x 3) feature vector obtained in feature extraction stage is used for testing the correct detection ratio of ME-ANN algorithm for RNA virus images in testing stage. So, 720 x 3 (12 x 60 x 3) feature vector is used in this stage. Obtained the detection accuracy of the proposed ME-ANN algorithm is given in Table 6.

Table 6. The correct detection rates for the proposed ME-ANN approach
(Tablo 6. Önerilen ÇE-YSA yaklaşımı için doğru belirleme oranları)

RNA Virus Types	Number of correct detected RNA virus	Number of Incorrect detected RNA virus	Percentage of correct detection rate (%)
PV	58	2	96.66
TV	57	3	95
FV	57	3	95
OV	60	0	100
BV	55	5	91.66
AV	54	6	90
RV	59	1	98.33
CV	60	0	100
REV	57	3	95
RHV	50	10	83.33
FLV	51	9	85
PRV	59	1	98.33
Total	677	43	94.02

Demonstration of the effectiveness of the proposed rotational and scale invariant features of automatic detection of RNA Viruses using Multi-entropy and Artificial Neural Network (ME-ANN) system is given in Table 6. As can be seen from Table 6, two of the twelve RNA virus images were classified with 100% correct classification rate. Moreover, the overall correct classification rate was 94.02%.

6. EXPERIMENTAL RESULTS AND DISCUSSION (DENEYSEL SONUÇLAR VE TARTIŞMA)

In literature, there are many pattern recognition studies [22, 23]. In these studies, different many feature extraction methods were used. In this study the center-edge changing and the calculating of entropies methods are used for feature extraction. There are some advantages of these methods such as invariant under rotation and scaling operations. Therefore, the considered RNA viruses can be detected from their images even these images are rotated or scaled.

In this study, the computer simulations on MATLAB environment are designed for fulfilling the system and testing the performance of the proposed automatic detection of RNA Viruses using Multi-entropy and Artificial Neural Network (ME-ANN) system. As given in Table 6, the developed system classified RNA viruses with a high success rate even rotation and scaling operations. The performance of the proposed system is 94.02 %. The main reason of incorrect classified RNA viruses is the similar shapes. A few of RNA virus images are uncertain identity due to the similar shape. As shown in these results, the preprocessing stage is most important part of this ME-ANN method for correct detection of RNA virus images.

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