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Analysis of DNA Gel Electrophoresis Images with Backpropagation Neural Network Based Canny Edge Detection Algorithm

Muhammed Kamil Turan (Corresponding author)
Karabuk University, Medicine Faculty, Department of Medical Biology
78050, Karabuk, Turkey
E-mail: kamilturan@karabuk.edu.tr

Abdullah Elen
Karabuk University, Vocational School, Department of Computer Technology
78050, Karabuk, Turkey
E-mail: aelen@karabuk.edu.tr

Eftal Sehirli
Karabuk University, Engineering Faculty, Department of Biomedical Engineering
78050, Karabuk, Turkey
E-mail: eftalsehirli@karabuk.edu.tr

Abstract

Gel Electrophoresis (GE) is one of the most used methods which separate nucleic acid and protein molecules according to electric charge, amount of them, molecule weights and other physical features. GE is used in many fields such as genetic, molecular biology and biochemistry. In this paper, Canny edge detection algorithm based on artificial neural network is used to separate and detect DNA bands automatically. Thus, GE analysis of gel electrophoresis period is realized and many properties of DNA bounds like intensity are extracted from digital images automatically.

Keywords: DNA Gel Electrophoresis, Canny Algorithm, Thresholding, Artificial Neural Network.

1. Introduction

DNA is a nucleic acid which carries genetic rules which are necessary for biological developments of all organisms and some viruses. Genetic information is inherited from generation to generation by DNA. It contains four nucleotides named as Adenine, Guanine, Thymine and Cytosine. The backbone of DNA consists of sugar and phosphate groups. There are ester bonds between sugar and phosphate group, hydrogen bonds among nucleic acids.

1.1 Agarose Gel Electrophoresis

Agarose GE is one of the most fundamental methods. The method is easy, fast-working and more advantages than other method which can't separate DNA bands sufficiently. It is difficult for molecules to move on gel pore during electrophoresis. Movement rates under electric field depend on the power of electrical field, the size and shape of molecules, relative hydrophobicity of samples, the ionic content and temperature of buffer where molecules move in [1]. Large size DNA molecules are blocked from passing through small pores in the gel. Thus, large size DNA fragments move less slowly than small size DNA molecules.

1.2 Movement of DNA Molecules in Gel

When voltage is applied on electrode, potential difference (E) is formed ($E = V/d$). At the same time, power on molecules $F = Eq$ is formed as well. While the power measured in terms of Newton provides charged molecules with moving through electrode, resistance caused by friction slows movements of charged molecules down. In addition to that, the functionalities of resistance are hydrodynamic magnitude of molecules, shape of molecules, pore magnitude in gel and buffer intensity [1].

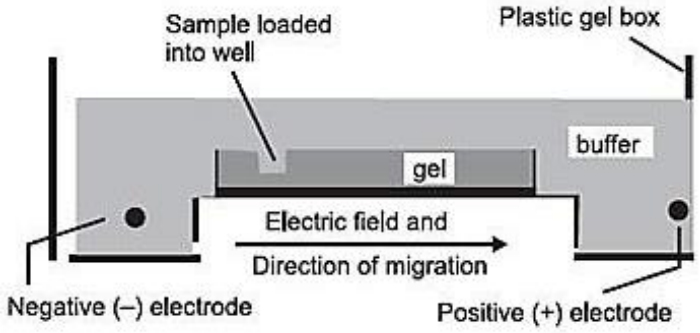

Figure 1. Movement of DNA Molecules in Gel [2]

The speed of the molecules loaded in the electric field (v) is a function of potential difference, load and friction power. At the same time, it expressed as $v = Eq/f$. Here f is the coefficient of friction.

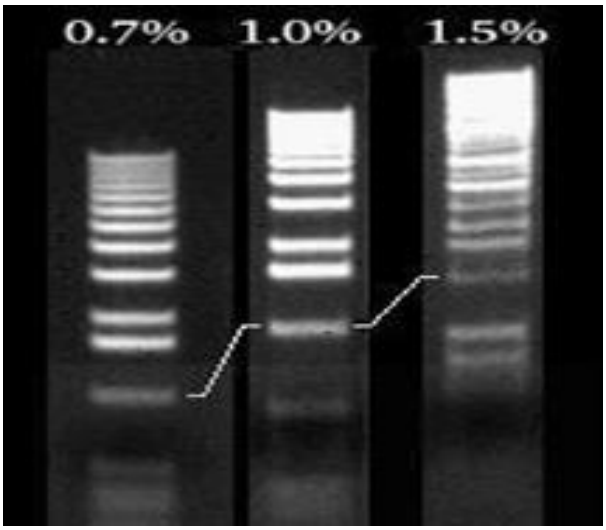

Figure 2. Separation of DNA molecules [3].

1.3 Ethidium Bromide and UV Light

Ethidium bromide is a chemical material which can be used in molecular biology laboratories to mark nucleic acids. These molecules appear as orange color molecules. Level of light increases 20 times more according to DNA molecules. As a result of that ethidium bromide is effective under UV light, ethidium bromide provides DNA molecules with appearing in gels. When DNA molecules which have ethidium bromide are being waited, mass of DNA molecules, induration of DNA molecules and movement of DNA molecules change in gels.

2. Related Works

Scales et al. have prepared a fully automatic thermo-gram analysis packet for diagnosis of breast cancer. In this study, canny edge extraction algorithm has been utilized to identify lower bound of body and breast. They have obtained 75% success for this study [4]. Liu et al. have studied to detect suspicious cancer cells on microscopic images. Canny algorithm has been used to identify edges of cancer cells and nucleus. Morphologic and color measure (RGB and HIS) techniques have been used to classify pathologic and normal cells. They have obtained 94% success for recognition of pathologic and normal

cells [5]. Qin and Zhang have studied on brain and skull images to extract their edges. When Canny algorithm has been compared with other edge detection algorithms such as Sobel, Roberts, Prewitt and Laplacian, they have determined that Canny gives better results on brain and skull images [6]. Chang et al. have developed a modified Canny edge detector to identify retina blood vessels in their studies. The modified Canny edge detector has based on dynamic hysteresis and threshold. They have concluded that Canny algorithm is very successful to identify retinal blood vessels [7]. Cheng et al. have developed a new image segmentation algorithm by combining GVF snake model with Canny operator. First of all, edge map has been obtained by Canny operator. After that, thin edges of objects on images have been obtained by using dilution techniques. Finally, exact edges of objects have been determined by using GVF snake model [8]. Bingrong and Mei have developed a half automatic algorithm to segment medical image series based on Canny operator and morphology method. All edges on images have been determined by Canny operator. Edges of closed objects have been determined by morphology method. According to results, they have proved that their developed algorithm is a fast and confidence algorithm to segment medical image series [9]. Tang has designed a neural network which can realize edge detection and compared the network with traditional edge detection methods like Prewitt, Sobel and Canny. According to results, while the neural network and Canny have had close results, other traditional methods have been insufficient [10]. Mei et al. have used Canny edge detection algorithm to detect brain tumor in their studies [11]. Chang et al. have used Canny operator to detect retinal blood vessels. The algorithm is successful at the rate of 94% [12]. Li et al. have used Canny detector to extract edges on medical dental images. It has been resulted that Canny operator is more successful than Harris edge detection method. Moreover, it has been foreseen that Canny may be effective to detect corners for 3D reconstruction [13]. Zhang and Gao have obtained successful results about edge extraction on medical images by combining Nonsubsampled Contourlet Transform with Canny algorithm in their studies [14]. When literature survey is done about Canny edge detection algorithm, it is obvious that Canny edge detection algorithm is more successful than other image processing edge detection algorithms for segmentation process. The main contribution of this study is to determine low and high threshold values for Canny algorithm automatically. By using artificial neural network, low and high threshold values are automatically obtained on agarose gel electrophoresis images. Thus, Canny algorithm is automated by means of artificial neural network in this study.

3. Materials and Methods

3.1. Materials

1% gel solution (500-10000 bp) for agarose gel electrophoresis is prepared. To capture gel electrophoresis images, a scientific camera has been used for this study. Properties of the scientific camera are shown in Table 1 below.

Table 1. Properties of the scientific camera

Parameters	**Values**
Image Sensor	½ inch, colorful 1.4MP CCD
Frame Rate	12.5fps@1360x1024, 15fps@680x520
Exposure Time	0.1ms~60mins
Color Filter	R, G, B
Scan Mode	Progressive Scan
Dynamic Range	60dB
SNR	52dB

3.2. Methods

3.2.1. Preprocessing Stage

The proposed algorithm is designed as a fully automatic system to determine edges of DNA cells on gel images. Fig 3 shows the flow chart of the preprocessing stage.

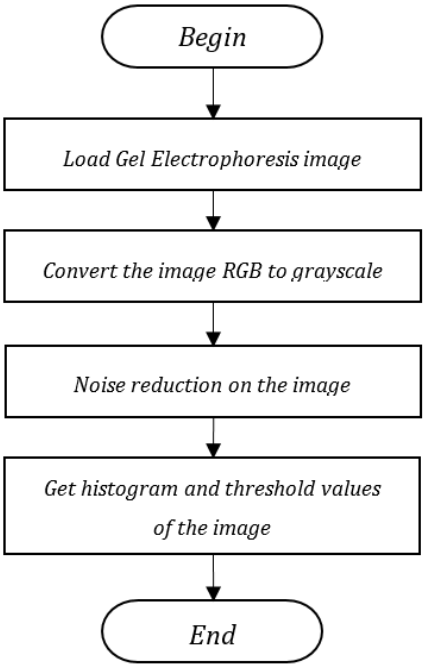

Figure 3. Flow chart for pre-processing stage

Images captured by the scientific camera are converted from RGB to grayscale. Noises which exist on images are removed by using Median Filter. Then, the threshold for edge detection methods other than the Canny operator Otsu method is determined. The reason for this is brought into a fully automated system for the purposes of designing. Canny algorithm for the high-threshold and low-threshold value will be estimated by improved artificial neural network.

3.1 Canny Operator

Marr and Hildreth's previous work derived from an improved edge detection algorithm. Best detection, which can give a clear answer and the optimal edge detection techniques with a good localization features. Making improvements in various forms, it is widely used in the existing image processing techniques [15].

Canny edge detection algorithm must be followed by a series of processing steps for implementation. Processing steps required for Canny operator is as follows [16].

1. Firstly smooth the image with a Gaussian filters.

$$G(x,y) = \frac{1}{2\pi\sigma^2}\, exp\left(-\frac{1}{2\sigma^2}(x^2+y^2)\right)$$

Where x is the distance from the origin in the horizontal axis, y is the distance from the origin in the vertical axis, and σ is the spread of the Gaussian and controls the degree of smoothing.

2. Compute the gradient magnitude and orientation using finite-difference approximations for the partial derivatives.

$$G_n = \frac{\partial G}{\partial n} = n \times \nabla G \qquad\qquad n = \frac{\nabla(G \times g)}{|\nabla(G \times g)|}$$

G_n is the first derivate of G and n is the direction, g is the image.

3. Apply non-maxima suppression to the gradient magnitude.

$$\frac{\partial^2}{\partial n^2} G \times g = 0$$

4. Use the double threshold (Low and High) algorithm to detect and link edges.

Block diagram belonging to Canny edge detection algorithm is shown in Fig 4. When 6 different edge detection algorithms are applied on a sample image, obtained results are shown in Fig 5. After threshold value of original image is calculated by Otsu thresholding method, the value is used for all algorithms except for Canny.

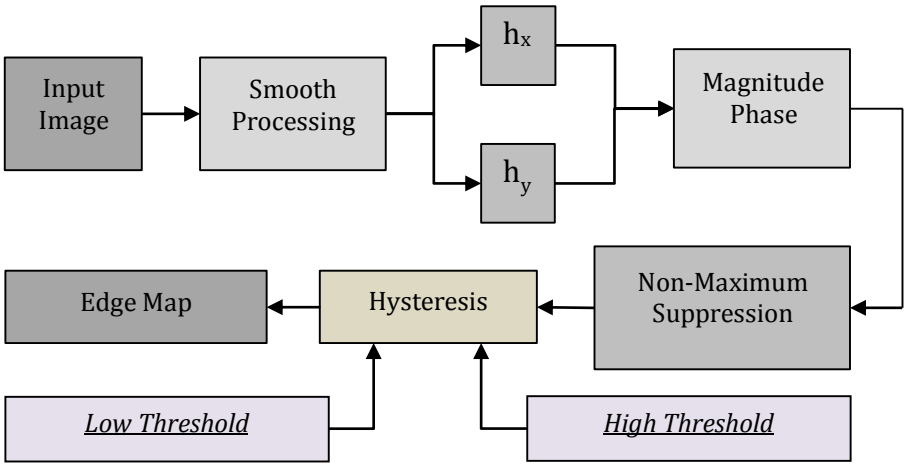

Figure 4. Block diagram of the Canny edge detection algorithm

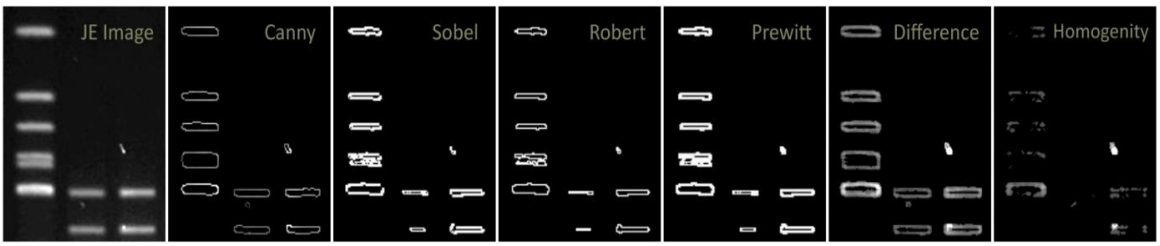

Figure 5. Obtained results after applying edge detection algorithms on a sample image

3.2. Connected Component Labeling

On the binary images, while pixels whose values are 0 are accepted as background, pixels whose values are 1 are accepted as objects. To determine shape and size of objects, connected component labeling (CCL) is used. Pixels are analyzed by looking at their 8-connectivity neighbors and connected pixels are given same label number [17].

Steps that are taken during CCL in this study are listed as below.

- Pixels are searched two times from left to right and from top to bottom.
- In the first search, pixels that don't belong to background are given label numbers starting with 1.
- Neighbors of a pixel that have a label number are checked. If all neighbor pixels belong to background, label number is increased by 1 for another object in the image. If neighbor pixel does not have ant label number, same label number is given.
- In the second search, it appears that components whose shapes are like U or E have different label numbers. In this case, the smallest label number are given to other pixels. Hence, connected pixels are given same label number.

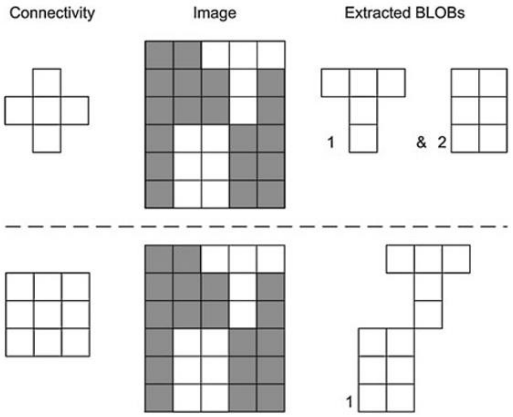

Figure 6. 4 and 8-connectivity. The effect of applying the two different types of connectivity [17].

In this study, an ANN is developed. 256 neurons are used for input layer. RGB values between 0 and 255 obtained by histogram are used for inputs. 2 neurons are used for output layer since ANN gives low and high threshold values for Canny algorithms. During test process of ANN, low and high threshold values are given manually. Properties of ANN are shown in Table 2 below.

Table 2. Properties of ANN

Parameters	**Values**
Inputs	256
Hidden Layer	8 neurons
Output Layer	2 neurons
Learning Rate	0.1
Momentum	0.5
Alpha	0.2
Error Limit	0.0001
Activation Function	Hyperbolic Tangent
Learning Method	Back propagation

** The High and Low thresholds of Canny operator were determined by Neural Network.*

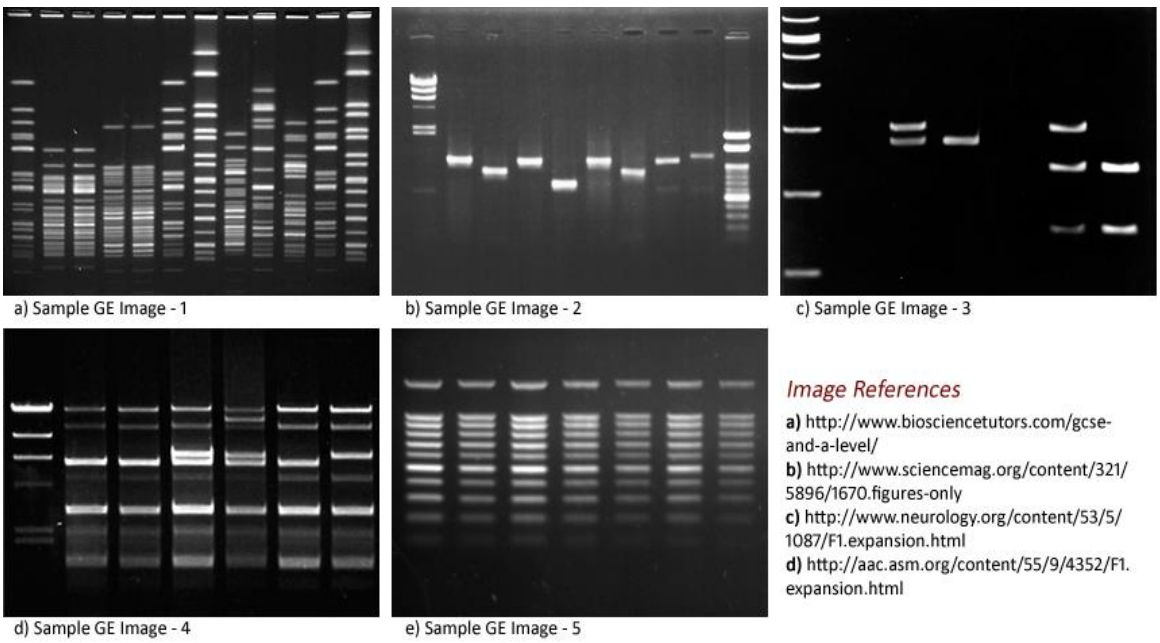

Figure 7. Sample gel electrophoresis images used in this study

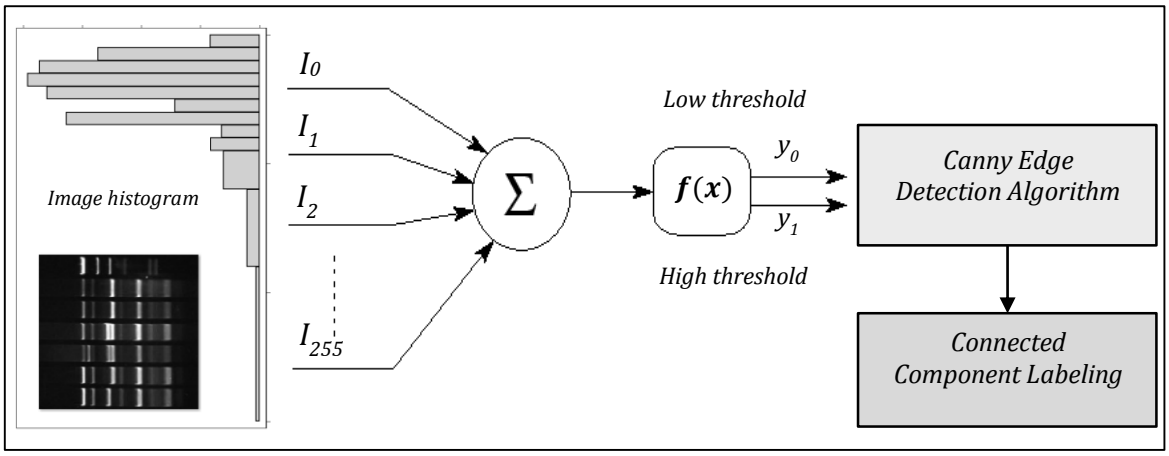

Figure 8. Structure of the developed ANN

Table 3. Number of the detected DNA molecules

Operator	Image-1	Image -2	Image -3	Image -4	Image -5
Canny + NN *	136 / 180	18 / 25	15 / 15	38 / 42	56 / 70
Robert	33 / 180	13 / 25	14 / 15	16 / 42	29 / 70
Sobel	44 / 180	18 / 25	14 / 15	18 / 42	41 / 70
Prewitt	46 / 180	13 / 25	14 / 15	18 / 42	37 / 70
Homogeneity	59 / 180	13 / 25	12 / 15	23 / 42	21 / 70
Difference	89 / 180	14 / 25	11 / 15	27 / 42	27 / 70

4. Conclusion

In this study, we have proposed an algorithm to detect DNA molecules on gel electrophoresis images. The proposed method is a backpropagation ANN based on Canny edge detection algorithm. Furthermore, the proposed algorithm is compared with other 5 different edge detection algorithms. Canny edge detection algorithm performs better and gives more accurate than other edge detection algorithms on gel electrophoresis images. The proposed method could not reach 100% success because intensity values of some DNA molecules are almost equal to threshold value of the images.

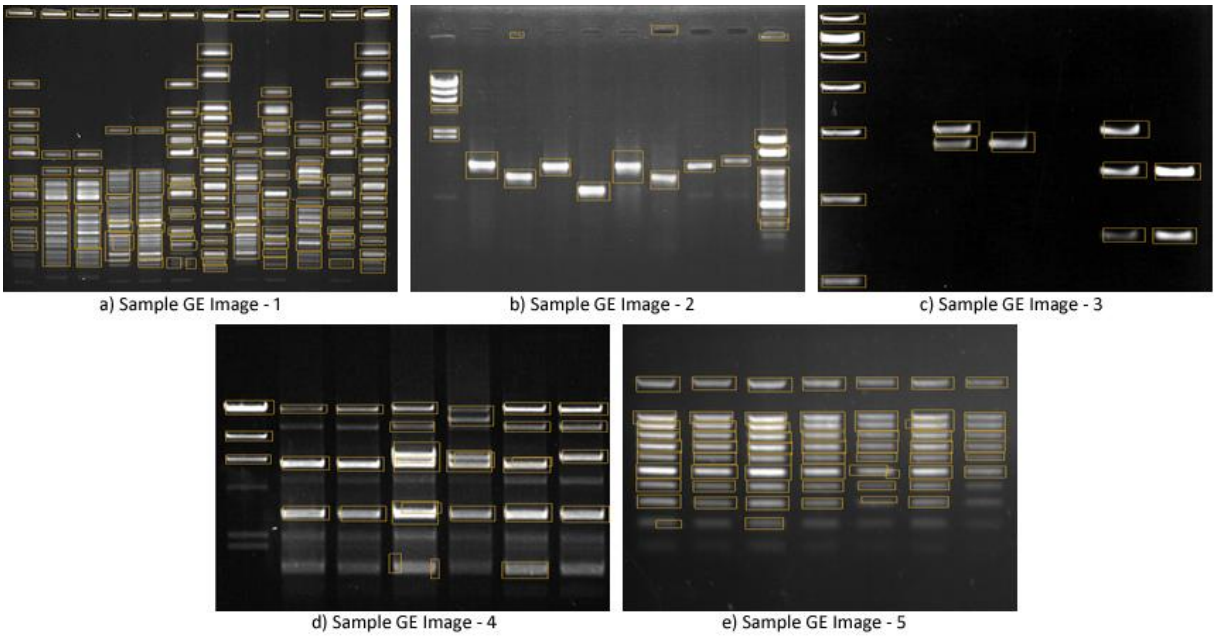

Figure 9. Detected DNA molecules on 5 sample gel electrophoresis images.

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