









Extraction of Cattle Retinal Vascular Patterns with Different Segmentation Methods

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ABSTRACT

In the field of animal husbandry, the process of animal identification and recognition is challenging, time-consuming, and costly. In Türkiye, the ear tagging method is widely used for animal identification. However, this traditional method has many significant disadvantages such as lost tags, the ability to copy and replicate tags, and negative impacts on animal welfare. Therefore, in some countries, biometric identification methods are being developed and used as alternatives to overcome the disadvantages of traditional methods. Retina vessel patterns are a biometric identifier with potential in biometric identification studies. Preprocessing steps and vessel segmentation emerge as crucial steps in image processing-based identification and recognition systems. In this study, conducted in the Kars region of Türkiye, a series of preprocessing steps were applied to retinal images collected from cattle. Fuzzy c-means, k-means, and level-set methods were utilized for vessel segmentation. The segmented vascular structures obtained with these methods were comparatively analyzed. As a result of the comparison, it was observed that all models successfully performed retinal main vessel structure segmentation, fine vessels were successfully identified with fuzzy c-means, and spots in retinal images were detected only by the level-set method. Evaluating the success of these methods in identification, recognition, or disease detection will facilitate the development of successful systems.

Keywords: Animal retina segmentation, CLAHE, Fuzzy c-means, K-means, Level-set

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1. Introduction

Segmentation and classification of retinal blood vessels in medical imaging have been extensively utilized in various fields of research [1]. Retinal blood vessels offer valuable insights for disease detection and identification studies [2]. By detecting diseases, it becomes possible to initiate the treatment process and facilitate the identification of existing ailments in animals. Moreover, early diagnosis allows for prompt intervention before the disease progresses further. Animal identification methods have yielded successful results in areas such as traceability and fraud prevention [3]. Traditional methods like earring, marking, and forging, which have been employed for cattle identification over the years, are associated with security vulnerabilities, susceptibility to fraudulent practices, and a negative impact on animal welfare, making them less preferred today. Advancements in technology have led to the increased adoption of identification processes utilizing biometric features, which are gaining popularity day by day.

The production and trade of animals and animal products are witnessing a steady increase, making it challenging to effectively monitor and track animals and their associated products. Animal tracking is particularly vital for ensuring safety and preventing the spread of diseases. Transparent and accurate animal identification processes must be implemented throughout an animal's lifespan to facilitate effective tracking. Biometric-based identification processes have gained prominence due to their superior success rates and enhanced security measures. The field of animal identification is witnessing a growing number of research studies as time progresses. Figure 1 illustrates the upward trend in the quantity of studies conducted in this domain across various years [4].

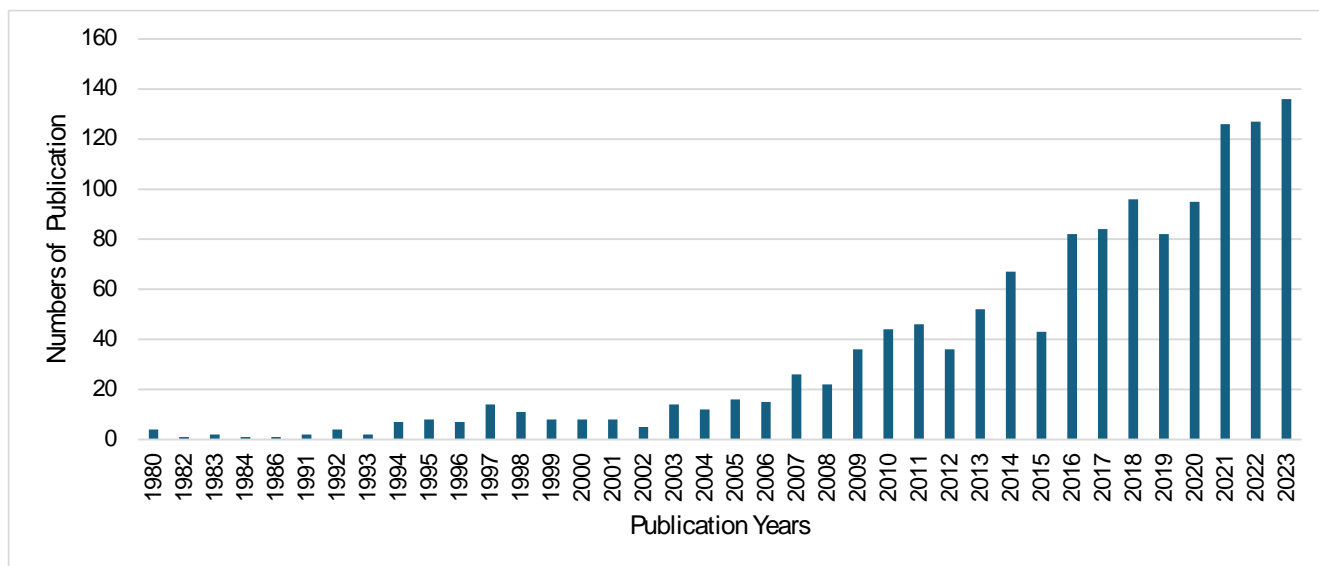


Figure 1. Representation of the Number of Studies Carried Out in the Field of Animal Identification by Years (1991-2023)

Biometric identification serves as the foundation of an individual's physical identity. Biometric features typically remain constant unless altered through medical procedures or injury, and they are inherently difficult to replicate exactly [5]. These methods, widely employed in human identification, are increasingly finding applications in animal identification as well. Biometric features in animals encompass characteristics such as retinal vessel patterns, nose prints, and iris patterns. During the selection process of a biometric feature to be implemented, it is essential to assess it against various criteria and make a decision based on the evaluation results. Here are the key points outlining the selection process for a biometric feature.

- **Universality:** The chosen biometric identifier should apply to all animals without compromising their confidentiality or health.
- **Uniqueness:** The biometric identifier must possess distinctive features that enable differentiation between two animals with the same characteristics.
- **Performance:** The identification criteria, such as accuracy, speed, and robustness, should be achievable. The required resources for attaining acceptable identification performance should also be manageable.
- **Circumvention:** The system's response to fraudulent techniques should be considered. The selected biometric identifier and the associated application should meet the requirements in terms of preventing fraudulent activities [6].

In this research, retinal vessel patterns, a biometric feature, were employed. Retinal vessel patterns exhibit a remarkable level of uniqueness and distinctiveness in both livestock and humans, allowing differentiation even among monozygotic (identical) twins [7]. Similar to medical image analysis, this study incorporated various preprocessing steps to analyze the retinal image. Subsequently, segmentation techniques were applied to achieve improved outcomes in the analysis.

Image segmentation plays a pivotal and indispensable role in the realm of medical image analysis, serving as a fundamental technique. Segmentation involves dividing an image into distinct parts or sections. The primary aim of image segmentation is to simplify the information in an image and make it readily analyzable and comprehensible. [8]. This simplification aids in analysis by assigning labels to individual pixels, enabling the identification and localization of objects within an image. Once the segmentation process is completed, the output consists of a collection of segments representing the entire image [9].

Extensive research has been conducted in the literature on the extraction of retinal blood vessels in both human and animal subjects. This section focuses on reviewing studies specifically related to the extraction of blood vessels in animals.

Mustafi et al. conducted a study introducing a system named RetIS for identification using goat retina images. The application of the suggested system involved the utilization of the Contrast Limited Adaptive Histogram Equalization (CLAHE) technique for image enhancement. The segmentation process employed the active contour algorithm, specifically the snake algorithm. Following segmentation, matching was performed utilizing the Hamming distance as a similarity measure [10].

The study focused on analyzing the temporal changes in retinal vessels using images obtained from dogs. Retinal images captured at specific time intervals were utilized to observe these changes. Two programs were employed to examine the variations in the vessels. The first program utilized the intersection points of two concentric circles, where a line was drawn from the midpoint of the retina, to observe temporal changes over time. In the second program, three major vessels were selected, and the changes in the angles between them were observed in subsequent images [11].

Several studies [1,12-14] have investigated retinal images of cattle and sheep. In all of these studies, the OptiReader device, patented in the United States in 2004, was employed to capture retinal images. This device enables automatic acquisition and storage of the vascular structure from retinal images, providing researchers with a convenient tool for their analysis.

In this study, after applying preprocessing steps to the collected cattle retinal images, three different methods were used to segment vessel patterns. These methods are Fuzzy c-means, K-means, and Level-set methods. The results of vessel segmentation using these methods were compared comparatively. It is noteworthy that detailed information about preprocessing and segmentation stages is not provided in retinal identification/recognition studies in the literature. Therefore, this study fills this gap by shedding comprehensive light on the preprocessing and segmentation steps, providing valuable information to researchers in this field.

2. Materials and Method

The retinal images used in the study were collected from cattle in the Kars region of Türkiye. Optomed Smartscope digital fundus camera was used to capture the retinal images. A total of 2430 retinal images were collected from the right and left eyes of 300 cattle in the study. In this study, retinal images collected from cattle were shared with the public via Kaggle (<https://www.kaggle.com/datasets/animalbiometry/cattle-retinal-fundus-images>). However, not all collected images were of sufficient quality to be used in image processing, so some were removed from the dataset. The images were removed from the dataset due to low quality, distortion caused by external factors such as lighting, and the inability to capture the retina clearly during the imaging process. The insufficient number of images (Animals with less than 2 retinal images were excluded from the study) obtained from the animals is also one of the reasons for the exclusion of the images. Sample retinal images that are of such poor quality that they cannot be used in the study are presented in Figure 2.

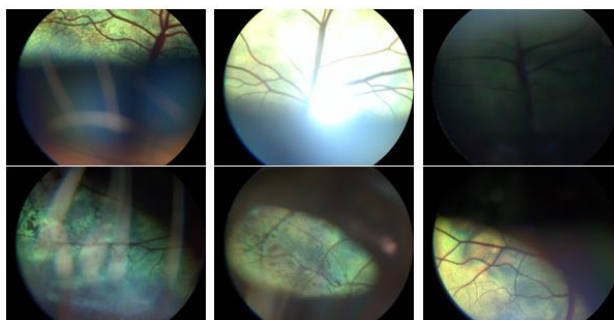


Figure 2. Sample Retina Images Removed from Dataset

As a result, a total of 1206 images belonging to 234 animals were used in the study. Figure 3 shows representative examples of high-quality retinal images used in this dataset. Notably, the images are characterized by a resolution of 1536x1152 pixels and are stored in the JPG format, a commonly employed file format for image compression and storage.

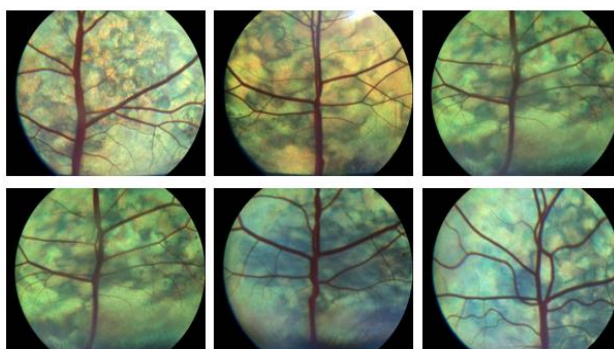


Figure 3. Sample Retina Images from Used Dataset

2.1. Preprocessing Step

Preprocessing steps play a critical role in achieving successful results in image processing methods. These steps help to improve the performance of the model by cleaning and optimizing the raw data. Operations such as reducing noise in images, improving contrast, and highlighting edges provide results with higher accuracy. In addition, these steps reduce the complexity of the model, providing a faster and more efficient processing process. For example, techniques such as resizing, graying, or normalizing images allow algorithms to work more effectively on the image. Therefore, accurate and effective preprocessing steps are a critical requirement in a successful image processing project.

Within our study, we organized the image preprocessing stage according to the flowchart depicted in Figure 9. This crucial phase encompassed several discrete procedures, including channel separation, extraction of the green channel, grayscale transformations, contrast limited adaptive histogram equalization (CLAHE), morphological operations, image extraction, and

noise filtering. For visual clarity, we handpicked three distinct images from our dataset to serve as illustrative examples. These selected images were subjected to the designated preprocessing steps, and the outcomes were meticulously recorded. To streamline computational efficiency while preserving image fidelity, we resized all images to a standardized resolution of 480x480 pixels before initiating the chosen preprocessing steps. By incorporating this resizing operation into our workflow, we were able to expedite the overall processing time while maintaining the integrity of the images.

2.1.1. Green Channel Extraction

As a pre-processing step to obtain retinal vessel patterns, one of the procedures involved separating the retinal image into its constituent color channels and subsequently extracting the green channel. The original image consisted of three color channels: red, green, and blue. By performing green channel subtraction, we focused on extracting the green channel from the image. This approach was chosen due to the observation that blood vessels exhibit higher contrast in the green channel compared to the other two channels. Additionally, the green channel offered a balanced representation without being under-illuminated or oversaturated, as opposed to the red and blue channels [15]. Figure 4 presents the resulting images after the extraction of the green channel.

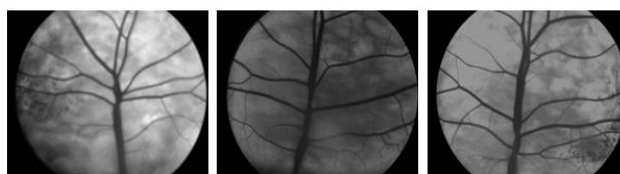


Figure 4. Three Different Sample Images Results of Applying Green Channel Extraction

2.1.2. Grayscale Conversions

Grayscale transformation is a commonly employed preprocessing step in various image processing techniques. Its primary purpose is to simplify algorithms and alleviate computational complexities. By converting an image to grayscale, the resulting representation offers easier visualization and interpretation compared to the original color image. Grayscale transformation effectively reduces the complexity associated with color information, allowing algorithms to focus on essential image features and patterns. This simplification facilitates the subsequent stages of image analysis and enhances the efficiency of processing algorithms.

2.1.3. Contrast Limited Adaptive Histogram Equalization (CLAHE)

Contrast Limited Adaptive Histogram Equalization (CLAHE) is a technique commonly employed to address the low contrast issues encountered in digital images, particularly in medical imaging [16]. In the context of medical imaging, CLAHE has demonstrated superior performance compared to other histogram equalization methods such as Adaptive Histogram Equalization (AHE) and standard Histogram Equalization (HE). CLAHE enhances contrast by imposing limitations on the contrast enhancement process, thereby mitigating the generation of unwanted noise. CLAHE achieves contrast limitation by defining a clip limit, which determines the maximum height of the histogram. This clip limit is adjusted based on the desired contrast requirements. By applying CLAHE following the gray-level transformations, the retinal vessels in the images become more discernible from the background. This enhancement, in contrast, enables improved visibility and differentiation of the vessels. Figure 5 showcases sample images where the CLAHE method has been applied, highlighting the effectiveness of this technique in enhancing image quality and vessel visibility [17].

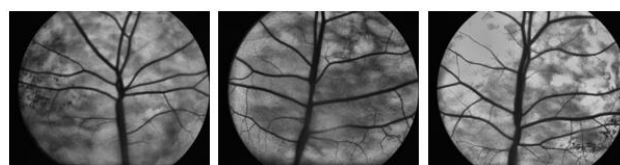


Figure 5. Three Different Sample Images with CLAHE Applied

2.1.4. Morphological Operations

When working with binary images, morphological operations are commonly employed to differentiate and extract specific components of interest [18]. Two primary processes involved in morphological operations are erosion and dilation. Erosion diminishes the size of objects present in the image, while dilation expands the area occupied by these objects. By combining these two operations, more intricate filtering operations can be achieved.

The process of dilation followed by erosion is known as opening, while erosion followed by dilation is referred to as closing. Opening operation smoothens object contours and eliminates thin protrusions. It has a similar contour-smoothing effect as closing, but it is particularly effective in removing small holes and filling gaps in the image [19]. These specific morphological operations, referred to as opening and closing, offer valuable tools for refining binary images, enhancing object shapes, and improving the overall quality of segmentation results.

To discern retinal vessels, morphological opening and closing operations were employed. These procedures aid in refining the segmentation of vessels in binary images. Figure 6 visually presents sample images where morphological opening and closing have been applied.

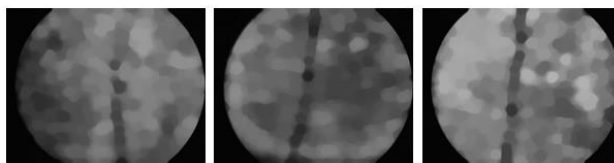


Figure 6. Example Images with Morphological Processes Applied

2.1.5. Image Subtraction

Image subtraction is a technique employed to isolate the objects of interest within an image from the background. In our study, an image extraction process was implemented to separate the vascular patterns from the surrounding tissues. Following the application of morphological operations, images enhanced with CLAHE were obtained. Subsequently, the image extraction procedure was executed to isolate the desired vascular patterns.

Figure 7 showcases sample images where the image extraction process has been successfully applied. These images demonstrate the effectiveness of the extraction method in isolating and highlighting the vascular patterns, thus facilitating further analysis and examination of the retinal vessels in the presence of minimal background interference.



Figure 7. Three Different Sample Images with Image Subtraction Applied

2.1.6. Noise Filtering

During image processing, filtering operations are commonly employed to yield valuable results. These operations serve to eliminate noise from images while preserving important details. In our study, filtering operations were performed to enhance the quality of the retinal vessel patterns.

Initially, the bitwise_not operation was applied, causing the image to be inverted at the bit level. This operation ensured that the retinal vessel patterns appeared as black, while the background was represented as white. This inversion facilitated subsequent analysis and visualization of the vessels against a clear background.

To further improve the image quality, the application of a median filter helped in noise elimination. The median filter effectively reduces noise by replacing each pixel's value with the median value within a defined neighborhood. This operation helps to maintain the important features and details of the vessel patterns while reducing unwanted noise.

Figure 8 depicts sample images where the noise filtering process has been successfully applied. These images demonstrate the efficacy of the filtering techniques in enhancing clarity and removing unwanted noise, allowing for more accurate analysis of the retinal vessel patterns.

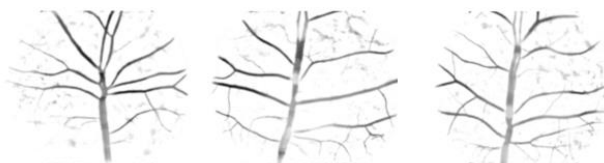


Figure 8. Three Different Sample Images with Noise Filtering Applied

2.2. Segmentation Step

In this section of the study, three distinct methods for segmentation are discussed: Level-set, Fuzzy c-means, and k-means methods. Each of these techniques offers a unique approach to segmenting retinal images. By applying these three segmentation methods to the selected sample images, the results are presented, showcasing the effectiveness of each approach in delineating the retinal vessels from the background.

2.2.1. Fuzzy C-means Method

The fuzzy c-means method is an unsupervised technique widely applied in various fields, including medical imaging, target recognition, and shape analysis [20]. In the domain of retinal image analysis, numerous studies have explored the segmentation of retinal images and the detection of blood vessels using the fuzzy c-means method [21-24].

Clustering is a commonly utilized approach for image segmentation, which aims to categorize patterns so that samples within the same group exhibit greater similarity compared to those belonging to different groups. The fuzzy c-means method represents one of the fuzzy clustering techniques that retain more information compared to traditional fixed clustering methods. This algorithm provides additional flexibility by allowing pixels to belong to multiple classes with varying membership degrees.

The objective of the fuzzy c-means algorithm is to partition a given set of pixels into a collection of "C" fuzzy sets based on a specific criterion. This algorithm operates by minimizing the objective function as depicted in Equation 1 [25]. The fuzzy c-means method leverages the mathematical optimization process to iteratively refine the assignment of pixels to different classes, resulting in the segmentation of the retinal image into distinct regions with varying degrees of membership to each class.

$$J_m = \sum_{i=1}^N \sum u_{i,j}^m \|x_i - c_j\|^2 \quad 1 \leq m < \infty \quad (1)$$

N represents the total pixel count, m is a real number greater than 1, $u_{i,j}$ is the membership degree of x_i in cluster j, x_i is the i th pixel in the cluster, the cluster's center is represented by c_j , while $\|*\|$ denoting the similarity between the center and the measured data. After taking the derivative of Equation 2, the equation obtained by using the Lagrange method is equal to zero, and Equation 3 is obtained. The M value indicates the coefficient determined according to the characteristics of the image. As the M value approaches infinity, the blurring increases and reaches full turbidity.

$$u_{ij} = \frac{1}{\sum_{k=1}^c \left(\frac{\|x_i - c_j\|}{\|x_i - c_k\|} \right)^{\frac{2}{m-1}}} \quad (2)$$

$$c_j = \frac{\sum_{i=1}^N u_{i,j}^m X_i}{\sum_{i=1}^N u_{i,j}^m} \quad (3)$$

2.2.2. K-means Method

The k-means method is a straightforward unsupervised technique commonly used in clustering problems. This method enables the classification of a given dataset into a predetermined number of clusters [26]. In the context of retinal image segmentation and blood vessel detection, the k-means method has been widely applied in numerous studies [27-28-29-30].

The k-means algorithm follows a set of steps:

1. The initial step involves defining the center points for each of the k clusters. It is crucial to position the center points as far apart from each other as possible.
2. Each data point's distance to the cluster centers is computed based on the dataset. According to this distance metric, each data point is assigned to the cluster that is closest to it.
3. After assigning data points to clusters, the average values of the cluster centers are recalculated, and the second step is repeated for all data points.
4. The iterations described in steps 2 and 3 are repeated until a specified number of iterations or a predetermined threshold level is reached.

Finally, this method aims to minimize an objective function. The objective function is shown in Equation 4. c_k denotes the object in the C cluster, y_i the center point of the cluster, K the number of clusters, S is the clustering partition of the set of entities, and the expression $\|.\|$ the distance metric.

$$W(S, C) = \sum_{k=1}^K \sum_{i \in S_k} \|y_i - c_k\|^2 \quad (4)$$

2.2.3. Level-Set Method

The level-set method, initially introduced by Osher and Sethian, is founded on the formulation of an appropriate equation of motion for evolving a boundary based on curvature effects [31]. While originally developed for solving physics and fluid dynamics problems, the level-set method has found substantial utility in the field of image processing in recent years.

The fundamental principle of the level-set method is to iteratively determine the boundaries of objects within an image gradually. At each stage of the method, contour curves are aligned with the object boundaries and adjusted accordingly. The objective is to minimize discrepancies between the contour curves and the actual object boundaries by employing smoothing techniques.

By gradually evolving the contour curves and minimizing differences between the curves and the object boundaries, the level-set method ensures the identification of the most accurate and appropriate object boundaries. This progressive refinement process facilitates robust and accurate segmentation results, allowing for precise analysis and interpretation of objects within the image.

Zero set of the function ϕ , active contour

$$C = \{(x, y) \mid \phi(x, y) = 0\} \tag{5}$$

is used to represent the contour and points inside/outside the contour are indicated as greater or less than ϕ . In Figure 9, the variation of the contour curves with the level-set method is given.

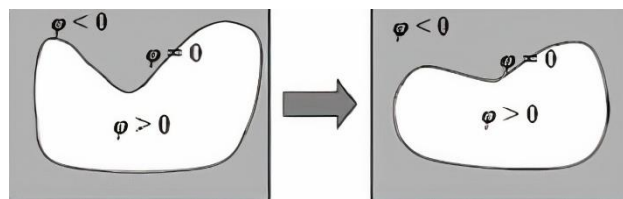


Figure 9. Contour curves change and representation of ϕ values [32]

3. Experimental Results and Discussions

To provide a comprehensive overview of the experimental workflow, Figure 10 depicts a flow chart diagram showcasing the sequence of preprocessing steps, segmentation methods, and subsequent operations applied to the images. This diagram serves as a visual representation of the entire process, illustrating the order and interconnectedness of the implemented techniques. A study was executed on a computer system equipped with an Intel(R) Core(TM) i7-11800H processor and 16 gigabytes of RAM. The coding phase was carried out using the Python programming language and the Anaconda Navigator platform.

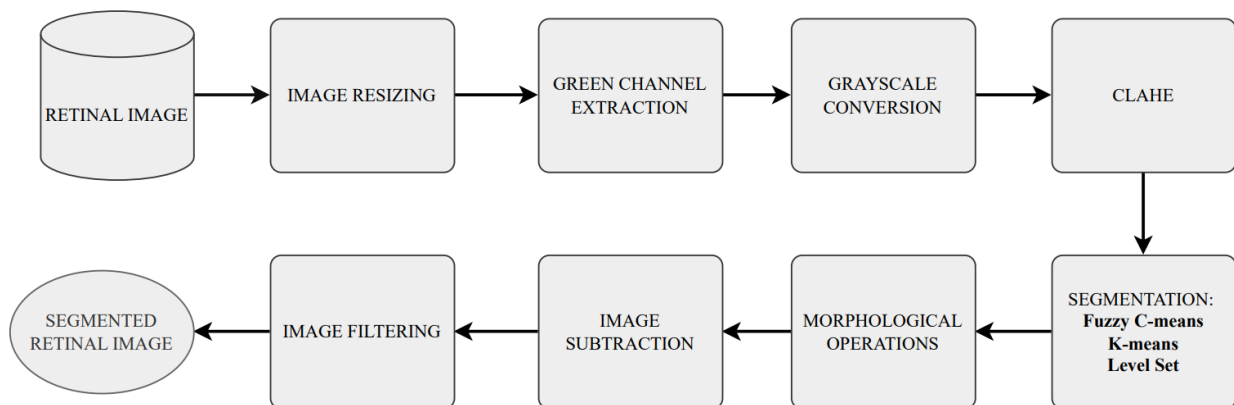


Figure 10. Flow Chart of This Study

In the conducted study, a series of preprocessing steps and distinct segmentation methods were applied to the acquired images. Table 1 presents the parameter names along with their respective values used in the employed methods. These parameters play a crucial role in determining the specific characteristics and behavior of each method during the segmentation process.

Table 1. Parameters and Values of the Methods

Method	Parameter	Value
CLAHE	clipLimit, tileGridSize	2.0, (9,9)
medianBlur	ksize	5
Morphological operations	kernel_size	(2,2) / (7,7) / (2,2)
fuzzy_c_mean	clusters, max_iter	2 / 4
k_mean	k, max_iter	2 / 4
level_set	mu, max_iter	0.1 / 200

The CLAHE method in this study employed two parameters: "clipLimit" and "tileGridSize". The "clipLimit" parameter defined the maximum allowed contrast enhancement, while "tileGridSize" determined the number of cells in the grid used for contrast equalization. The "ksize" parameter in the medianBlur method represented the size of the kernel used for blurring or smoothing the image. For morphological operations, the "kernel_size" parameter indicates the selected size of the kernel employed in these operations. In the fuzzy_c_mean method, the parameter "clusters" denoted the desired number of clusters, while "max_iter" represented the maximum number of iterations to be performed during the algorithm's execution. In the k_mean method, "k" refers to the number of clusters to be generated, and "max_iter" represents the maximum number of iterations to be executed. In the level_set method, "mu" denoted the determination rate of the contours, influencing their evolution, and "max_iter" parameter sets the upper limit for the number of iterations performed by the level-set algorithm.

The segmentation results obtained are given in Figure 11. In Figure 11, the segmentation results created by the original image, fuzzy c-means, k-means, and level-set methods are given on the same line. In different rows are the results of images taken from different animals.

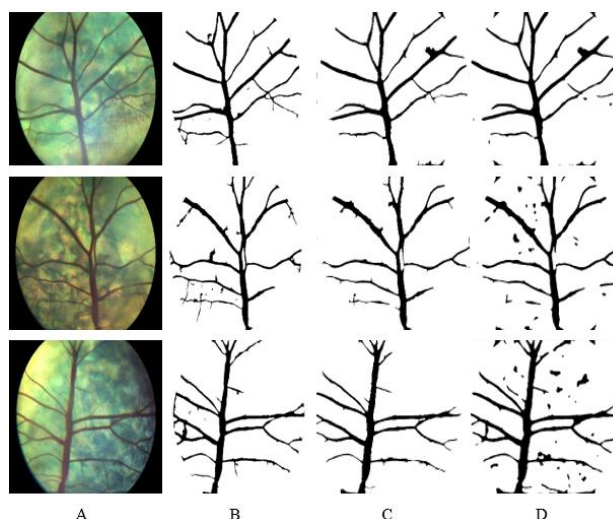
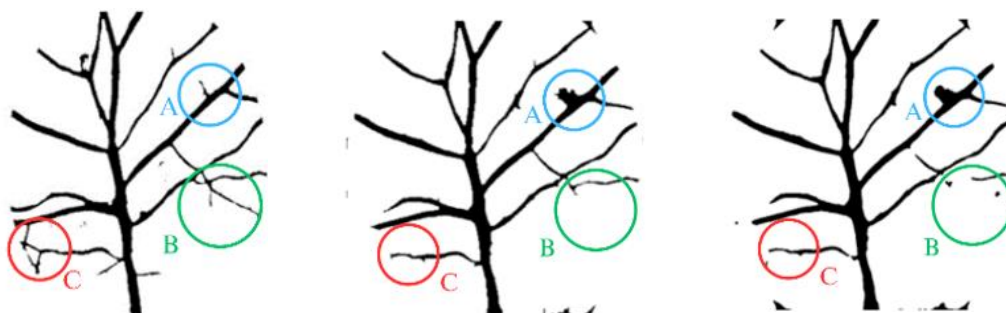


Figure 11. Retinal images for (A) Original and Segmented from (B) Fuzzy c-means, (C) K-means, and (D) Level-set method

Differences between segmented images obtained with three different methods (Fuzzy c-means, K-means, and Level-set) have been identified in the study. Marking these differences will help the segmentation results to be interpreted more successfully. In Figure 12, there are marked representations of some differences in the images obtained as a result of the methods. In the Figure, parts belonging to the same region are encoded with the same-colored circle.

Sample 1



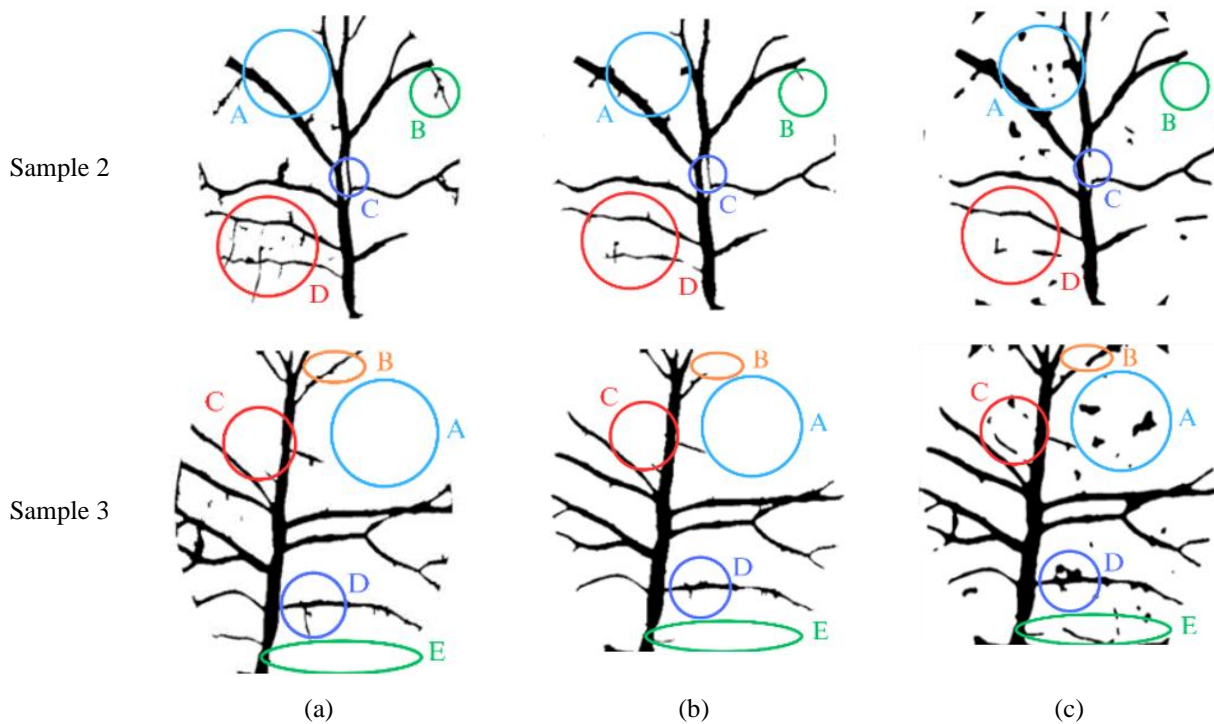


Figure 12. Comparison of Segmentation Results for (a) Fuzzy c-means, (b) K-means, (c) Level-set Method

Figure 12 - Sample 1: When examining the vessel regions marked with three different colors, it is observed that fine vessels are better detected with the fuzzy c-means method in region A enclosed by the blue circle, while it is observed that the k-means and level-set methods could not fully capture the structure of fine vessels. Similarly, when examining the vessels marked as B (green circle) and C (red circle), it is seen that fine vessels are better detected with fuzzy c-means, while other methods could not fully extract these vessels.

Figure 12 - Sample 2: In this retinal image, four regions have been marked; it is observed that the level-set method detects the spots in the background in region A. Upon examination of the original image in Figure 10, it appears that there are spots in the background of the retinal image. It is important to segment these spots if they are associated with a disease. When areas B, C, and D are examined, it is again observed that fine vessels are more clearly obtained by the fuzzy c-means method.

Figure 12 - Sample 3: In the last example, five different areas have been marked with circles on the retinal image. Upon examination of the marked areas, it is evident that the level-set method successfully segmented the background spots in areas A, C, and E. While the entire fine vessel in region B was obtained using the fuzzy c-means method, only a part of the vessel could be extracted with the k-means and level-set methods. In area D, the fine vessel was well detected with the fuzzy c-means method; however, it is observed that the spots were more effectively removed with the level-set method.

As a result, although there is no significant difference in main vessel segmentation among the methods, fine vessels have been better extracted using the fuzzy c-means method, and the spots in retinal images have been detectable with the level-set method. It is also worth investigating which method could be more successful in identification, recognition, or disease diagnosis, and this should be evaluated as a research topic.

In existing literature studies with animal retinal images, the segmentation process is often performed automatically using specific pre-built software. However, in our study, the results obtained from various segmentation methods were compared and evaluated. This approach allowed for a more comprehensive analysis and assessment of the different techniques employed in the segmentation process. However, it is known that applications such as artificial intelligence and image processing are limited in the field of animal science [33, 34]. Nonetheless, there is a noticeable trend of increasing popularity in these areas with each passing day. In this study, fundamental information that will increase interdisciplinary studies has been presented to researchers.

4. Conclusion

In this study, the aim was to present researchers with a detailed exploration of preprocessing and segmentation steps for the development of identification, recognition, or disease diagnosis systems based on cattle retina patterns. Particularly, these processes are crucial for the transition from traditional animal identification methods, such as ear tagging, to biometric-based systems.

Cattle retina images collected from the Kars region of Türkiye were used in this study. In the preprocessing step, the green channel extraction method was applied to enhance the visibility of veins, followed by applying the grayscale transformation

to the image, addressing low contrast issues with CLAHE, applying morphological operations, obtaining clear vessel structures with image subtraction, and finally, applying noise filtering for noise removal and background elimination. Following preprocessing, the vascular patterns in these images were segmented using fuzzy c-means, k-means, and level-set methods. Upon comparative examination of the segmented vessels, it was observed that all models effectively segmented retinal main vascular structures, while the fuzzy c-means method was more successful in extracting fine vessels and the level-set method was more successful in removing speckles from retina images.

This study contributes to the existing literature by providing a detailed implementation of preprocessing steps for animal retina images and offering a comparative analysis of segmentation techniques. In contrast to many existing studies based on automatic software, our approach includes a systematic comparison and evaluation of various methods. In the future, the next step will be to evaluate the effectiveness of these methods in identification, recognition, and disease diagnosis. Additionally, by highlighting the importance of interdisciplinary research in the context of artificial intelligence applications in animal sciences, this study provides a valuable perspective for future endeavors in this developing field.

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Authors' Contribution

Authors contributed equally to the study.

The Declaration of Conflict of Interest/Common Interest

No conflict of interest or common interest has been declared by the authors.

Data Availability

The collected Cattle Retinal Fundus Image dataset is available on Kaggle, <https://www.kaggle.com/datasets/animalbiometry/cattle-retinal-fundus-images>

The Declaration of Ethics Committee Approval

The study was approved by the Kafkas University Animal Experiments Local Ethics Committee (Protocol number: KAÜ-HADYEK/2024-123).