Detection of *Escherichia Coli* Bacteria by Using Image Processing Techniques

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Abstract: - Recently, image processing has proven itself as a fast and reliable technique in research in medicine and biology. Bacterial colony separation is an important and time-consuming process in studies in the field of microbiology. Bacteria counting is usually carried out by the naked eye or even by Coulter counter machines, which are based on the rather expensive electric field measurement method. In this study, image-based enumeration of Escherichia Coli over the colony morphology in the petri dish was investigated.

In the experimental study, 4 different bacteria from the Enterobacteriaceae family were planted on petri dishes containing Eosin-methylene blue agar (Merck, Darmstadt, Germany). Escherichia Coli colony characteristics were determined by digitizing planted bacterial petri images. For the study, counting was done with the interface developed in MATLAB R2013a. After the classification criteria were determined, the method was tested on new petri dishes and successful results were obtained.

Key-Words: Escherichia Coli, MATLAB GUI, Image Processing

I. INTRODUCTION

Image processing is developing and widely used with the advancing technology today. With these developments, solutions are sought with image processing to the problems we encounter in every field. Image processing technologies show successful results in the fields of medicine, security, production, education and science. One of the difficulties in image processing is obtaining a healthy image to be processed. It is aimed to reach the desired result by processing the obtained image either with the ready-made functions provided by the program being studied or with the algorithms we have developed. Various programming languages have been developed for these studies [1,2].

Matrix Laboratory (MATLAB) has become one of the most preferred application environments in studies in the field of image processing with the convenience and opportunities it provides. In the MATLAB Image Processing Toolbox, there are ready-made mathematical algorithms for all kinds of applications, as well as the programmer can perform the function he needs. The image to be processed in the MATLAB Image Processing Toolbox is perceived as a matrix when it is imported into the environment. And the algorithms to be applied are applied on the matrix. It is possible to write code in MATLAB, as well as to test many applications by preparing simulations of operations. In studies in the field of medicine and biology, image processing techniques provide both fast and reliable results in reaching the conclusion of the data [3].

Escherichia coli is the most frequently isolated bacteria in routine stool cultures, although it is found 100 times less than obligate anaerobic bacteria in the normal intestinal flora. It plays a role in preventing the colonization of pathogenic microorganisms. Since the 1960s, information has begun to increase that some E. coli strains are also pathogenic in the intestine. E. coli is an important opportunistic pathogen that can cause a wide variety of infections outside the intestine [4].

E. coli is a gram-negative rod-shaped, non-spore-shaped bacterium, approximately 2-6 μm in length, 1-1.5 μm in width, with flat and rounded ends. Some origins are encapsulated. They are mostly motile and lactose-positive bacteria that can grow at 37-44˚C in general use media. They can form gas from carbohydrates, but not from starch. They form indole from tryptophan. IMVIC tests are (+ + - -). They do not create H2S in TSI. Except for some species that cannot break down lactose, E.coli bacteria form colonies that give a pink-red color on McConkey agar and a purple and metallic sheen on EMB (Eozine Methylene Blue) agar [5].

In academic studies in the field of microbiology, in the field of food, in the field of cosmetics, bacterial counting is important and time-consuming. The total bacteria count in such studies can be done with high-cost machines called BioCounter, as well as by applying various methods and tests with human power. In particular, the parameters considered in the microbiological control of water and food are quite high.

Examples of these are; we can give Bacillus cereus count, total coliform count, Escherichia coli, mold and yeast count, lipolytic bacteria count, mesofolic anaerobic bacteria search, psychrophilic bacteria count. Often defined as "total bacteria" or "total viable bacteria" in international trade, it is actually the total number of aerobic mesophilic bacteria [6].

II. LITERATURE REVIEWS

Li Xiaojuan, Chen Cunshei, Yv Ziyi wanted to classify novel bacteria by extracting their morphological features from their microscopic images. It is aimed to write appropriate algorithms for determining the edges of bacteria in microscopic images, removing noise, and distinguishing similar objects in the best way. The results were compared with the NMRC database, and they observed that the method gave reliable and fast results [7].

The aim of the study conducted by Sigal Trattner, Hayit Greenspan, Gabi Tepper, Shimon Abboud; To develop the toolbox that will automatically select bacteriophages in the microbiology database by using image processing techniques and statistical modeling techniques. Bacteriophage-type methods have been used for the identification and profiling of samples, for example in Staphylococcus bacteria. Thus, the use of manpower is minimized, data consistency and time savings are achieved. With the statistical method created in this study, automatic, objective and powerful data analysis can be performed [8].

Hio Chon Ngiap, Ian A Watson, Ruikang K Wang, Glenn Ward investigated the possibilities of finding the best colony count with image processing systems in their study. The pace of development of the field of microbiology has been slow in history. Today, with the development of image processing techniques, it is tried to minimize human errors that may occur during sample analysis and to accelerate data acquisition. For example, in commercial productions, there are machines that can count the colonies of bacteria planted in agar culture in petri dishes. Great advances have been made in *Escherichia coli* counting using OPTIMAS (commercially developed software package for general image processing applications). With this system, effective solutions have been presented to various applications [9].

In the study conducted by Manuel Guillermo Forero, Filip Sroubek, Gabriel Cristóbal, it was aimed to identify the tuberculosis bacteria by looking at their shape and color-based characteristics. Tuberculosis and mycobacteria are serious diseases that can be controlled with early diagnosis. Various techniques are used to find bacilli in saliva image analysis.

However, saliva analysis is time-consuming and the error rate that may occur from human power is high. Image processing techniques are a good framework for improving manual viewing of samples.

The aim of the new autofocus algorithm and new bacillus detection technique, which is the subject of this article, is to increase the high precision of the analysis of saliva samples and to minimize the time consumption. This technique is based on a combination of simple edge detection methods over a fixed shape-based color channels. Some properties were determined by examining samples prepared from bacilli. Clustering technique was used while evaluating the classification results obtained using the ROC analysis structure [10].

Robert L. Parry, Taiwai Chin, Patricia K. Donahoe wanted to perform cell colony counting in petri dishes with the help of computer. It has been emphasized that it is very difficult to count cells that are very close to each other. Counting cell colonies with a light microscope is a tedious process. On the other hand, colony counting will be difficult unless the strict double-blind (a method often used in medical experiments, both the subject group and the treatment group do not know which is the real drug and a placebo (ineffective) drug). With the computer software application presented here, precise, reproducible, cell colony counting can be done with minimal user bias. This application is based on Apple IICX computer system and image software and Apple Scan.

Colonies 24 petri dishes were prepared properly for high quality scanning. Each colony was well counted after the scans were transferred to the image. Success in counting was achieved thanks to the light microscope [11].

J. Marotz, C. Lübbert and W. Eisenbeiß aimed to develop a software with a system that can automatically and efficiently count colonies in petri dishes. It is the number of colonies in the water, which is the traditional method in the field of microbiology to reveal the quality of drinking water. This tedious counting process is done manually in traditional methods. Computer-based counts are put forward by using digital images obtained from the images of Petri dishes. This method works with high accuracy thanks to its high-specification and effective object identification algorithm. This process includes internal control so that the evaluation of images in series can be done automatically by the system.

Flatbed scanner or CCD cameras can be used to obtain the image. Samples in the standard preparation process in the laboratory are evaluated according to the appropriate algorithm. Objective improvements in colony count and quality are aimed at the reliability of this method. High performance and effective solution has been obtained with the algorithm specialized in finding the objects in the Petri dish. This study was carried out on a total of 150 petri dishes and the petri dishes were counted manually by 3 different people. The same petri dishes were counted with the created system. This system has achieved 90% success [12].

In this study, it was aimed to distinguish *Escherichia Coli* from other bacteria and to find the total number of colonies of *Escherichia Coli* bacteria in the petri dish. This will save time. Manpower requirement will be minimized and the cost of required biochemical tests will be reduced.

III. MATERIAL AND METHOD

Escherichia coli is found in mammals and birds as intestinal bacteria. It is present in a balanced manner with the flora in intestinal activities. With the decrease of immunity, it mixes with the tissue or blood, has the feature of infection and causes diarrhea. In this study, eosin methylene blue (EMB) agar (Merck, Darmstadt, Germany) was cultivated by plate spreading method, and images of 15 petri dishes were used.

The cultures used in the experiment were produced by incubating in nutrient broth (Merck, Darmstadt, Germany) medium at 37ºC for 24 hours. The densities of the cultures at the end of the logarithmic growth times were adjusted to 105 using the McFarland turbidity constant followed by serial dilutions in physiological saline. In order to be used in the study, the petri dishes to be imaged were inoculated with no dilution (sample dilution 100), 1/100 dilution (sample dilution 10-2), and 1/10,000 dilution (sample dilution 10-4). This procedure was repeated twice for each dilution of each sample.

Figure 1. Sowing phase

In the interface created with eight buttons and two axes, each button executes a different code and presents the visuals in one of the axes. The image of the interface created below is shown in Figure 2.
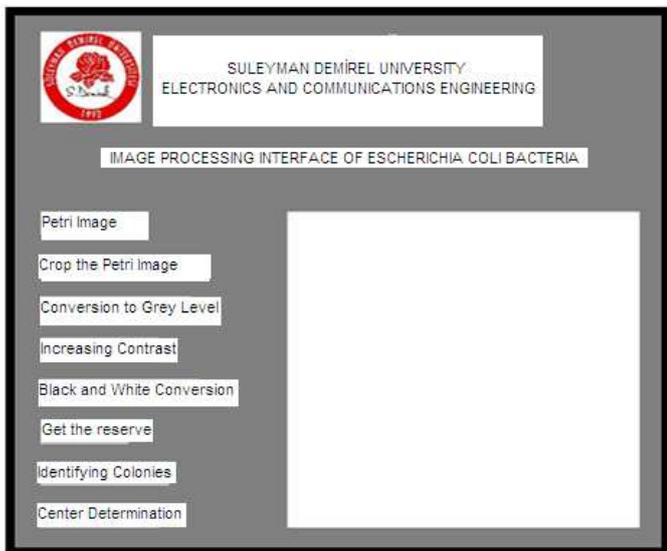

Figure 2. Interface created in MATLAB GUI

IV. RESEARCH FINDINGS

A. Importing the Image to be Processed into the Program

The captured photo is taken in color from the MATLAB folder to the interface created in jpeg format. An example of the image taken is shown in Figure 3.
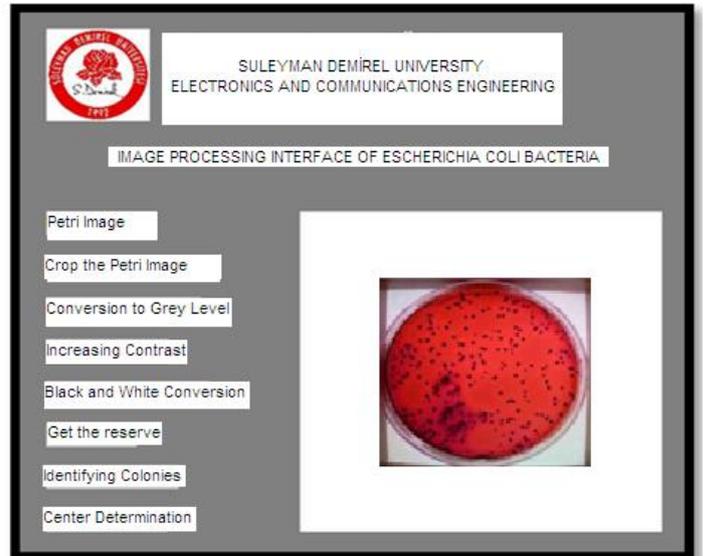
Figure 3. Color JPG photo taken from the microbiology laboratory

B. Removing Petri from Image

Due to the large size of the image taken in color form, it prolongs the running time of the program. At the same time, it causes the formation of unwanted noise at the edges. For this reason, the parts to be processed by the petri dish program are removed from the picture and the result in Picture 5-3 is obtained. The algorithm of this operation is the midpoint of the x and y coordinates of the obtained image. These coordinates are assigned as the center of the circle and the radius is determined. While drawing a circle with a definite center and radius, the part inside the radius is left in its original form, and the part outside the radius is turned black.
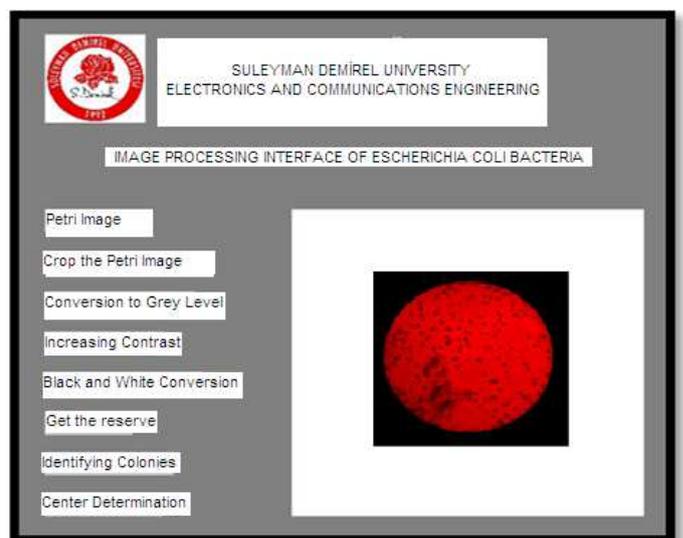

Figure 4. Cropped image output

C. Converting Colony Image to Gray Image

Each pixel has R, G, B values. Due to the high resolution of these values, the processing of the picture takes quite a long time. This time is shortened when the image is converted to gray level. Therefore, the image taken in color form is converted to gray level form. To perform this operation, the rgb2gray function of the MATLAB image processing toolbox is used. This function places the mixture of three different colors, red, green, and blue, into a gray level color between 0 and 255. The petri image converted to gray level shape is shown in Figure 5.
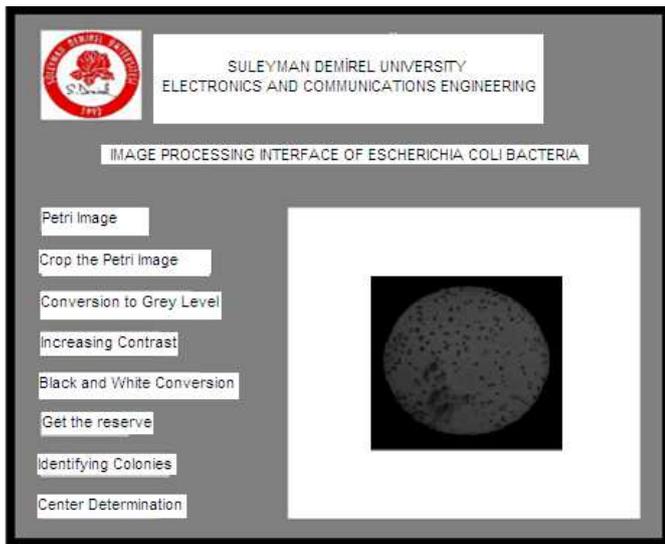

Figure 5. Petri photo converted to gray level

D. Increasing Brightness

By adding brightness or blurring to the picture, it helped to create a distinct difference between the background and the objects on the picture. Thanks to this process, objects are separated from the background and colony loss is prevented. The petri image obtained as a result of this process is shown in Figure 6. This action adjusts the image density values or color map. While converting the density values in the original image to new values, it reconstructs the original image according to the low and high intensity values. This provides an increase in contrast in the picture.
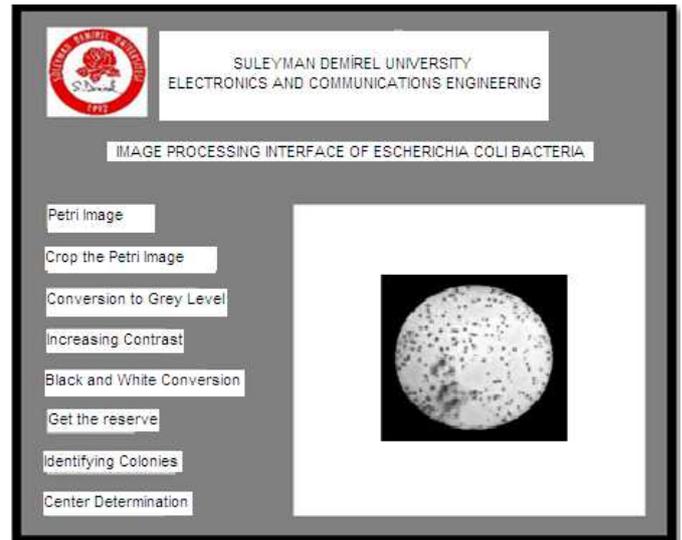

Figure 6. Petri dish with enhanced contrast

E. Black and White Conversion

Colonies will be equal to 0 and background 1 according to the threshold value determined by the program to separate the objects on the image from the background. For this process, the im2bw function from MATLAB image processing tools is used. When converting the picture to black and white form, the threshold value, called threshold, must be determined. Threshold value is determined with graythresh command. This threshold value takes values between 0-1. In the picture, those below this value are expressed as black, and those above it are expressed as white. Black ones are denoted by 0 and white ones by 1.
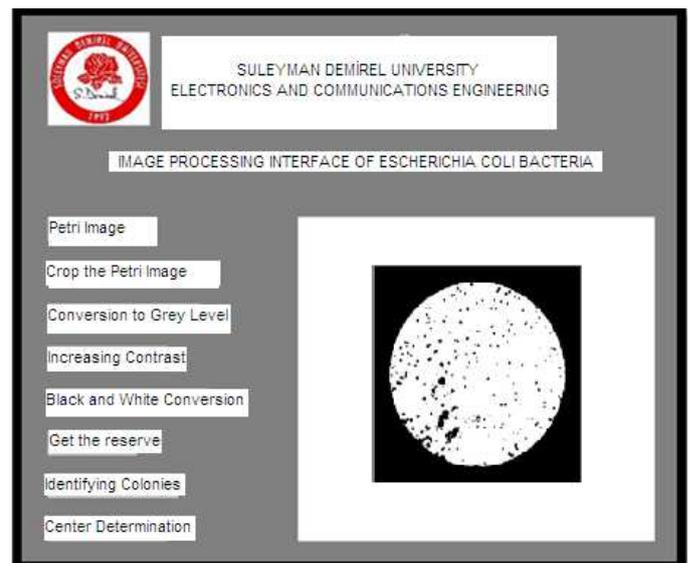

Figure 7. Petri photo converted to black and white

F. Reversing

In order for the image to be processed, it must be inverted. This happens when objects take the value 1 and the background 0. The result of this process is shown in Figure 8.
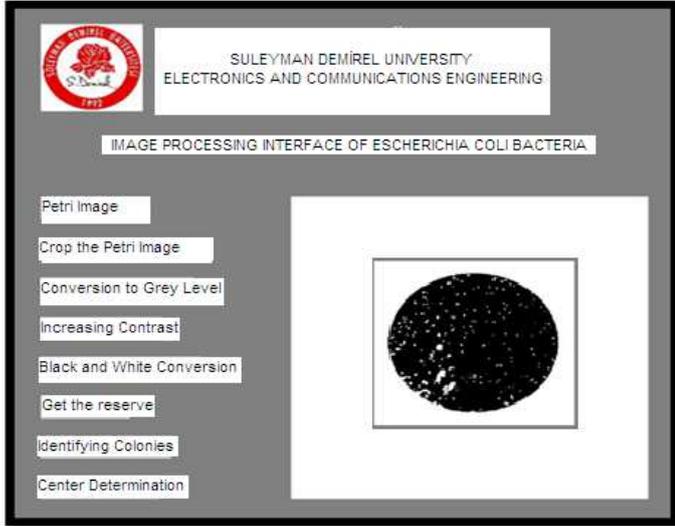

Figure 8. Inverted photo

G. Identifying Colonies

After this process, the imclearborder function, which is a MATLAB image processing toolbox function, is used to remove unwanted noise from the picture that touches the edges of the petri dish. This function operates according to the value assigned to it. After deleting the objects touching the edges of the picture and eliminating the small areas, our picture image takes its appearance in Figure 9.
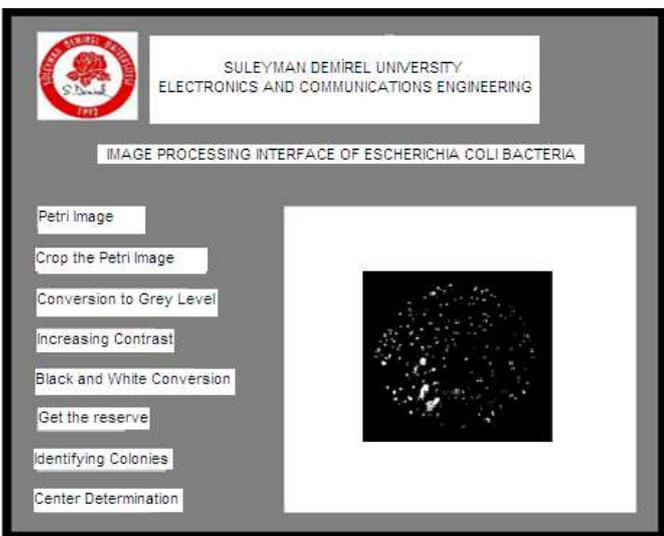

Figure 9. Petri dish with cleaned edges

H. Drawing the Boundaries of the Labeled Colonies

From the MATLAB image processing toolbox, which allows plotting the boundary values of the tagged colonies. This command helps to determine the boundaries of objects as hollow or full. the 'noholes' value allows the boundaries of colonies to be determined without leaving gaps in them. Returns the B and L values of the matrix values of the colonies whose boundaries have been drawn. In Figure 10, the boundaries of the colonies have been drawn and an image has been obtained.
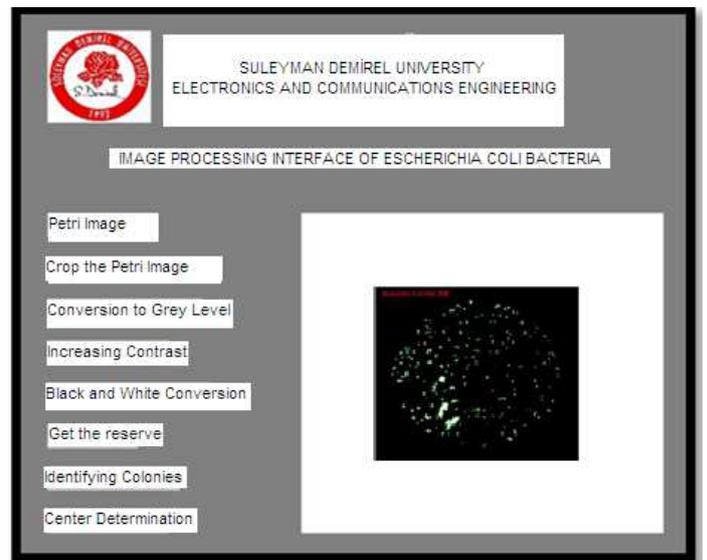

Figure 10. Petri photo with colony boundaries drawn

V. CONCLUSIONS AND DISCUSSION

This study, it will alleviate the work done especially by those working in the field of microbiology and the audience in the field of microbiology academy. Devices capable of counting colonies have been developed and are being used in this field. But the cost of these devices is quite high, in addition, these devices only provide the user with information about the number of colonies, and do not provide information about the type of colony. However, the costs of tests for the identification of bacteria, as well as the probability of human error in colony counting studies, are quite high. In this system, the information about the number of colonies and the number of colonies of *Escherichia coli* will be presented to the user, which will save time, reduce the cost of chemical tests, and will be an alternative to high-cost devices.

With the help of the developed computerized image processing system, more reliable and healthy data will be presented in the colony count. Using the colony counting system from various petri dishes;

- Speed is provided.
- Savings have been made on the intensity and cost of chemical tests.
- It has minimized the possibility of errors.
- It has become an alternative to expensive devices.

In this section, it covers the procedures to be performed to determine the morphological characteristics of each labeled, borderline colony and to diagnose which of them meet the characteristics of Escherichia coli. At this stage, there is a need for image processing. For this reason, an application has been developed in the MATLAB program. This application developed is to determine the properties of the colonies contained in the image that have undergone input filtering processes.

Various parameters have been determined in determining the characteristics of colonies. In the studies conducted on bacteria in the field of microbiology, it has been revealed that each bacterium can have different properties from each other, as well as have common properties. The december structure of bacteria belonging to the Enterobacteriaceae family has been studied and a certain range of criteria has been determined for each morphological feature of Escherichia coli. If the morphological characteristic values remain within this criterion range when the colonies are december, the program will show the result that this colony belongs to the *Escherichia Coli* bacterium. Density-based properties and shape-based properties were used to diagnose the colonies in the image. All of these components are calculated for all colonies.

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