MAS Journal of Applied Sciences
Uygulamalı Bilimler Dergisi
ISSN: 2757-5675
masjaps.com

OPEN ACCESS

DOI: http://dx.doi.org/10.5281/zenodo.10673417

Research Article

Developing a Solution Density Analysis Program with Image Processing Technique

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Received: 20.11.2023 **Accepted**: 25.12.2023

Abstract

Image processing is a computer program that can be used in many industrial applications, integrated with computers. Image processing consists of a series of operations. These processes begin with the capture of the image, pre-analysis preparations are made with image enhancement techniques, and object detection processes are performed. In this study, information is given about the interpretation of the Elisa microplate prepared in laboratory environments using image processing techniques. The aim of the study; It offers alternative analyzes and syntheses through the image processing program, apart from the ongoing existing analysis methods. It is thought that the analysis of the relevant samples using the image analysis program may be an alternative to the existing detection methods. In addition, it has been shown that thanks to the program, it will be possible to reach more precise information in a shorter time. The fact that the analysis and synthesis stages of the preparations are laid out in a systematic way and the pictures taken from the preparations are easily analyzed clearly show us that image processing techniques have a very important place in the field of health, as in every field in today's technology world.

Keywords: Image proccessing, elisa microplate, image analyse program

1. Introduction

Image processing is used in many technological fields (robotics, traffic, industry, medicine, military, geography). It also allows the production of many algorithms following the processing of images. The main purpose of image processing is; improving distorted, old and noisy images and sharpening and observing objects that are difficult to see and need to be detected. This study aims to establish a structure that is more economical and requires less resource use in order to ensure that scientific and academic studies continue without interruption by creating another detection-analysis system as an alternative to the results obtained from photometric measuring devices. For this purpose, the usability of image processing techniques is aimed as an alternative to the photometric measurement method. Generally, image processing has been used in research in different branches of science. The most current of Elisa image processing research is the article by Koekoeh Santoso and his colleagues titled "Comparing Antibody Testers After Vaccination In Dogs Using Image Processing Techniques" in 2021. In this study Santoso; took the image of the ELISA plates using a desktop scanner and showed that the results obtained as a result of image processing had a close reading to the results obtained from the ELISA reader (Santoso et al., 2022). In the article titled “Optimization of a Paper-Based ELISA for a Human Performance Biomarker” researched by Richard C. Murdock and his colleagues; Using a digital camera or Windows- or Android-based tablets that are as efficient as a camera, they imaged paper-based ELISAs (P-ELISAs), performed image analysis with MATLAB, and produced response curves that were highly correlated with target biomolecule concentration (Murdock et al., 2013). Additionally, Douglas J. Soldat and his colleagues wrote in their article titled “Microscale Colorimetric Analysis Using a Desktop Scanner and Automated Digital Image Analysis” in 2009; They show that digital image analysis of a scanned microplate image can replace a spectrophotometer for several common quantitative microscale procedures (Soldat et al., 2009). In the studies of Erkan Türker and his friends; a computer program that can make a more objective evaluation with numerical data such as average bead diameter, number of beads, and number of beads per unit area has been developed (Turker et al., 2017). In the studies of Jesper Ø. Hjortdal and his friends; found digital image processing useful for in vitro biomechanical studies of the cornea (Hjortdal and Jensen, 1995). In the articles of Federica Zanca and her friends; have shown that image processing has a significant impact on the detection of microcalcifications in digital mammograms (Zanca et al., 2009). This study aims to establish a structure that is more economical and requires less resource use in order to ensure that scientific and academic studies continue without interruption by creating another detection-analysis system as an alternative to the results obtained from photometric measuring devices. For this purpose, the usability of image processing techniques is aimed as an alternative to the photometric measurement method.

2. Material and Method

In this study, we used data from the Elisa plate with 96 wells. Images of the plates were obtained from Dicle University Central Laboratory Elisa Department. There are 8 control and calibrator and 88 patient samples in the plates and their images are shown in Figure 1.

2.1. Analysis with the Classical Method

With the help of ELISA test, many infectious diseases such as HIV/AIDS, Measles, Rubella, Mumps, Hepatitis B or pregnancy can be diagnosed. Reading the results of various biological samples in the microwells of the plate reader is generally used for the purpose of observations of physical, chemical and biological aspects of studies. Researchers and scientists use the Elisa reader device to evaluate various

samples in their microwells (Anonymous, 2022). Photometry measures the absorbance of a substance in a solution; it can be expressed as the difference between the amount of light coming out of the light source and the amount of light falling on the current detector. If this process is done using filters, the device is called a photometer; if it is done through slits or prisms, it is called a spectrophotometer. To determine the solution concentration, it is calculated by comparing the amount of light absorbed by a solution of known value with the amount of light it absorbs (optical density/OD). Measurements in spectrophotometers include the concentrations of solutions in existing samples. For this purpose, the wavelength appropriate for the detection of the substance in the solution is selected. The result is reached after comparing it with the absorbance (A) or Optical Density (OD) of another sample of known concentration and performing the necessary mathematical operations. Or the absorbance values of the solutions are compared with the absorbance value of a solution whose absorbance is assumed to be zero. For this purpose, a certain wavelength is chosen. Afterwards, the result corresponding to the absorbance of the solutions can be found through this graph (Anonymous, 2022). With the help of the photometric measurement method, the OD value of each well of the plate placed in the Triturus brand Elisa analyzer was determined. After the photometric calibration of the device is performed with the help of calibrators, the OD values and positive (patient) or negative (not patient) status of the samples are evaluated with positive and negative controls. Celiac disease is a disease that occurs in individuals with a genetic predisposition as a result of consuming gluten-containing grains in the diet. It is also an autoimmune, chronic inflammatory and systemic disease (Fasano, 2005). In a multicenter study conducted in our country, the prevalence of celiac disease was found to be 0.47% (Dalgic et al., 2011). The presence of Celiac disease was investigated in the samples loaded on the plates. Tissue transglutaminase IgA test is performed to diagnose celiac disease. For IgA testing, the Elisa method is an option. Figure 1. shows the raw image of the 96-well Elisa plate to be analyzed.

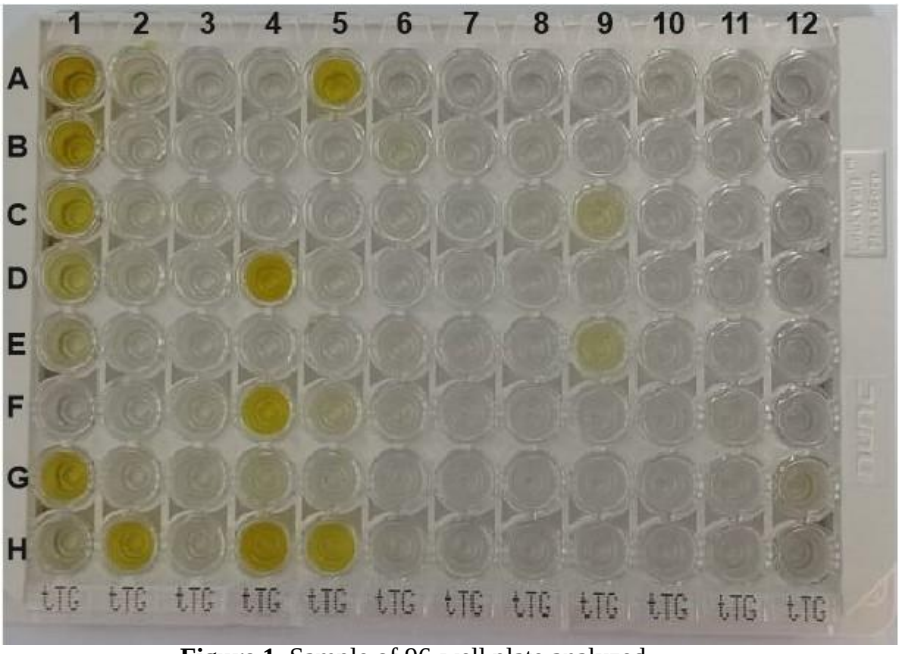

Figure 1. Sample of 96-well plate analyzed

6 wells on the plate between A1-F1 are the calibrator, well G1 is the positive control and well H1 is the negative control. The remaining 88 wells contain patient samples. Optical Density (OD) values of each well are shown in Table 1.

Table 1. Optical Density (OD) values of the samples-I

Well Number	OD Value	Well Number	OD Value	Well Number	OD Value	Well Number	OD Value
A1	2,862	**A2**	0,087	**A3**	0,057	**A4**	0,07
B1	2,053	**B2**	0,052	**B3**	0,046	**B4**	0,069
C1	1,13	**C2**	0,072	**C3**	0,116	**C4**	0,051
D1	0,501	**D2**	0,099	**D3**	0,068	**D4**	4,367
E1	0,246	**E2**	0,081	**E3**	0,065	**E4**	0,046
F1	0,023	**F2**	0,042	**F3**	0,117	**F4**	1,863
G1	1,483	**G2**	0,085	**G3**	0,094	**G4**	0,169
H1	0,156	**H2**	1,537	**H3**	0,092	**H4**	2,385
A5	2,209	**A6**	0,052	**A7**	0,046	**A8**	0,05
B5	0,073	**B6**	0,159	**B7**	0,063	**B8**	0,067
C5	0,054	**C6**	0,083	**C7**	0,032	**C8**	0,096
D5	0,095	**D6**	0,04	**D7**	0,04	**D8**	0,059
E5	0,025	**E6**	0,088	**E7**	0,061	**E8**	0,028
F5	0,207	**F6**	0,078	**F7**	0,028	**F8**	0,032
G5	0,07	**G6**	0,057	**G7**	0,05	**G8**	0,044
H5	1,233	**H6**	0,054	**H7**	0,062	**H8**	0,041
A9	0,042	**A10**	0,092	**A11**	0,077	**A12**	0,045
B9	0,038	**B10**	0,04	**B11**	0,052	**B12**	0,046
C9	0,312	**C10**	0,037	**C11**	0,031	**C12**	0,055
D9	0,055	**D10**	0,046	**D11**	0,047	**D12**	0,067
E9	0,345	**E10**	0,035	**E11**	0,017	**E12**	0,022
F9	0,037	**F10**	0,039	**F11**	0,072	**F12**	0,034
G9	0,036	**G10**	0,04	**G11**	0,04	**G12**	0,146
H9	0,039	**H10**	0,04	**H11**	0,048	**H12**	0,061

2.1. Analysis with Image Processing Program

The first thing in the works created with the help of image processing techniques is to take images from the camera. On the obtained images, image pre-processing steps are applied and feature extraction of the focused objects is performed. Different methods are recommended in studies aimed at detecting or recognizing objects. Studies on fast and effective object recognition by determining simple features of objects and using these features (Viola and Jones, 2005), complex background extraction in order to make images clearer and more suitable for analysis (Hussin et al., 2012), shape recognition, color recognition, edge and corner recognition, statistical pattern recognition, various methods such as template matching are used (Sonka et al., 2014). In order to precisely analyze the color changes in each well of the plate passing through the stages of the Elisa method, it is necessary to first take a photograph of the plate in the best possible quality and upload it to the Carestream MI image processing program in our system. With the help of the program, image settings such as brightness, blurriness and contrast can be easily adjusted. We can also resize the image to the size we want before analysis. After converting the image to grey-scale, the area to be measured for density is determined and selected on the image. A reference shape is created according to the size of the wells in the selected region (the image of each well is close to an ellipse). These automatically reproduced ellipses, which are created in Figure 4.2, are placed with 1 ellipse on each

of the 96 wells, and the number of pixels, brightness, density, etc. values of each of the areas under the ellipses are shown through the program. Apart from this, another method that can be used is the edge detection method. Edge detection is a method that attempts to detect sudden changes in the gray levels of an image by identifying sharp discontinuities in an image (Shrivakshan, 2012). The process performed here is not to manipulate the pixel values of the image, but to measure the density of the target areas by making the necessary markings.

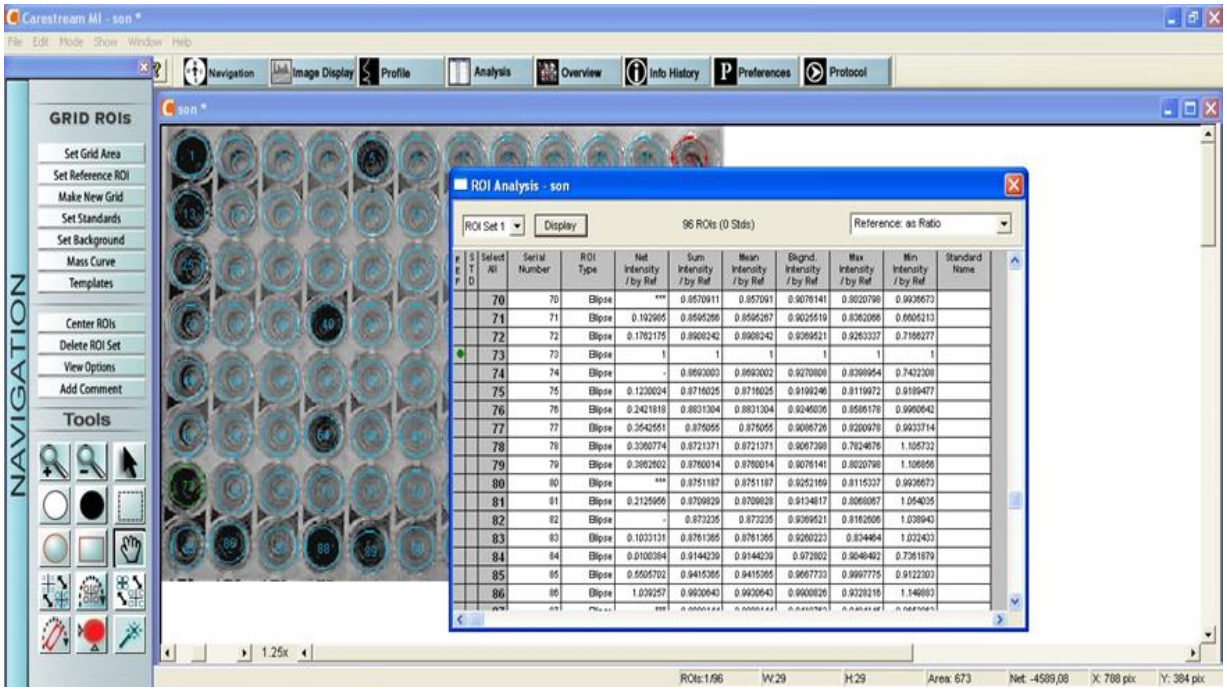

Figure 2. Image processing program analysis screen

Optical density (OD) determines the amount of matter in a substance by measuring the amount of light it transmits, respectively, and is expressed as:

$$\text{Optic Density} = C\left(\log_{10}\frac{A-B}{I}\right) \quad (1)$$

In Equation 1;
C = A user-defined constant can be used to equalize concentration changes in a given experiment. (Default case of C is = 1).
A = Maximum observable intensity (white point).
B = Minimum observable intensity (black point).
I = The intensity value for each pixel in the source image.

The general operating procedures of the image processing program are given in detail in Figure 3.

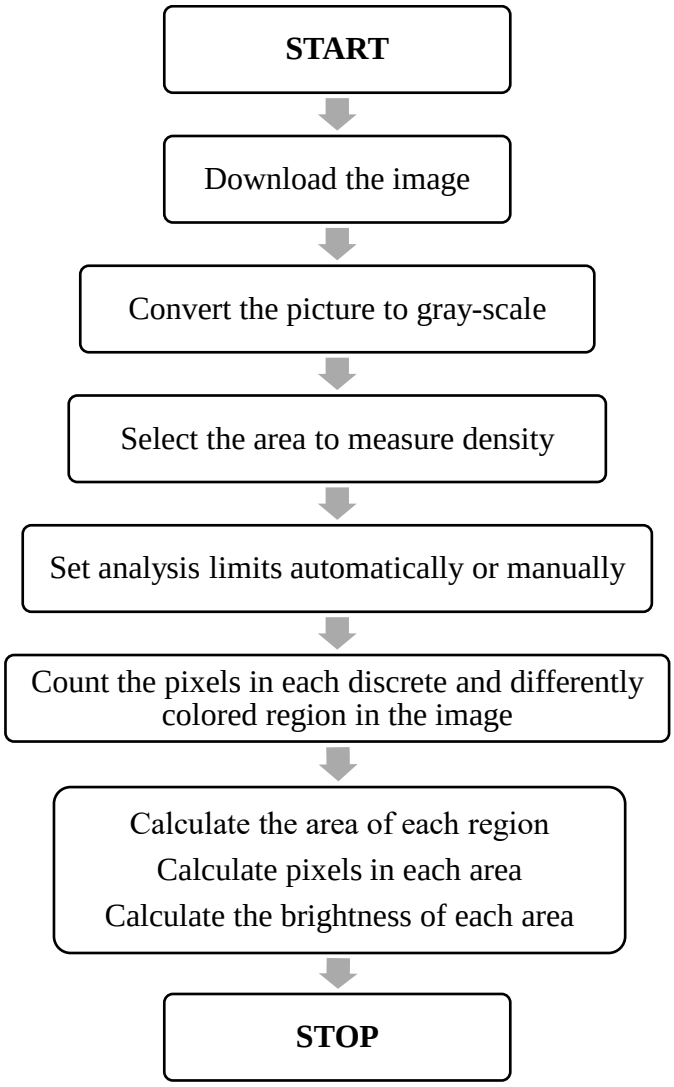

Figure 3. Image processing program general flow chart

3. Results and Discussion

The samples in each well on the Elisa plate can be read precisely with the image processing technique. We can take the positive control value in the plate (well no. 73 in Table 2.) as a reference value. According to the density value of this control, whose value is determined in advance, the densities of all other samples can be easily calculated. Values below the positive control indicate negative (not sick), values above indicate positive (patient). The densities of the areas indicated by the marked regions are given in Table 2.

Table 1. Density (number of pixels) values of the wells

Well Number	Density	Well Number	Density	Well Number	Density	Well Number	Density
1	1,9819	**25**	0,7802	**49**	0,269867	**73**	1
2	0,4135	**26**	0,2889	**50**	0,431955	**74**	0,4490
3	0,3919	**27**	0,2651	**51**	0,363036	**75**	0,1230
4	0,1430	**28**	0,6576	**52**	0,481995	**76**	0,2421
5	1,1511	**29**	0,7089	**53**	0,600188	**77**	0,3542
6	0,2511	**30**	0,3182	**54**	0,310748	**78**	0,3360
7	0,2794	**31**	0,2857	**55**	0,221280	**79**	0,3862
8	0,3114	**32**	0,3801	**56**	0,113307	**80**	0,6083
9	0,2005	**33**	0,7820	**57**	0,551520	**81**	0,2125
10	0,1137	**34**	0,2028	**58**	0,106890	**82**	0,2002
11	0,2595	**35**	0,8226	**59**	0,617121	**83**	0,1033
12	0,6198	**36**	0,1828	**60**	0,145872	**84**	0,0100
13	1,3737	**37**	0,4261	**61**	0,155526	**85**	0,5505
14	0,2449	**38**	0,1575	**62**	0,221524	**86**	1,0392
15	0,2545	**39**	0,1069	**63**	0,405283	**87**	0,4938
16	0,3668	**40**	2,8082	**64**	1,286057	**88**	1,6046
17	0,4378	**41**	0,7143	**65**	0,561002	**89**	0,8104
18	0,2197	**42**	0,2363	**66**	0,523465	**90**	0,4276
19	0,3283	**43**	0,2474	**67**	0,430579	**91**	0,8459
20	0,4180	**44**	0,1677	**68**	0,213946	**92**	0,1946
21	0,2780	**45**	0,4138	**69**	0,155171	**93**	0,4773
22	0,4523	**46**	0,5099	**70**	0,286329	**94**	0,5841
23	0,2982	**47**	0,2189	**71**	0,192984	**95**	0,5081
24	0,4482	**48**	0,3113	**72**	0,176217	**96**	0,3719

In Figure 4, with the help of the program, the rainbow image of the picture was selected and the minimum (purple), maximum (red) and gamma settings in the color scale were adjusted.

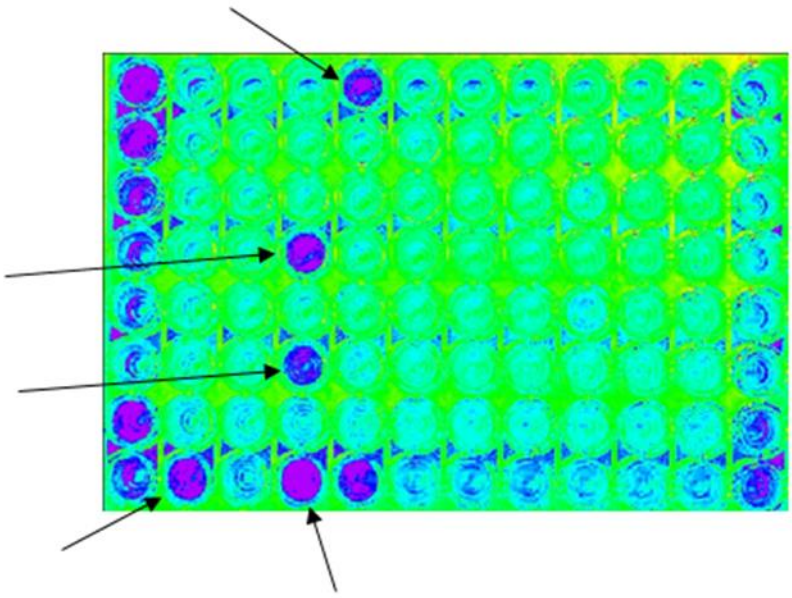

Figure 3. Example image with rainbow color palette

In Figure 5, the original image is shown in greyscale. Positive values were clarified after brightness, contrast and gamma adjustments were made.

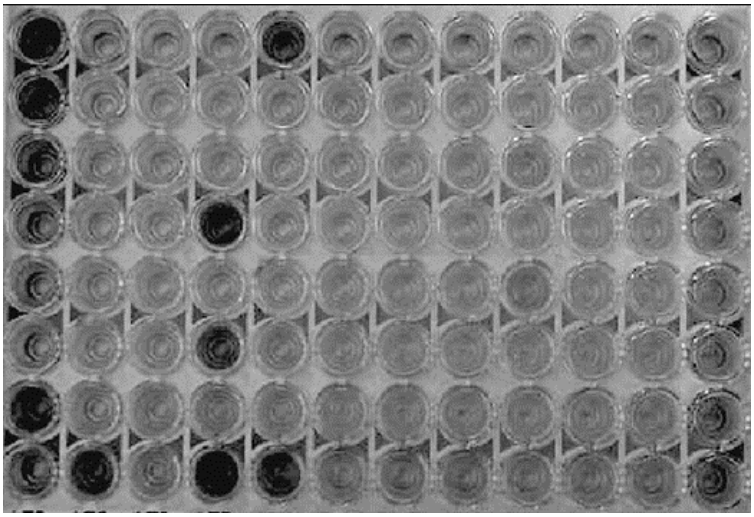

Figure 4. Example greyscale image

Wells A1, B1, C1, D1, E1, F1; contain values that are generally used for device calibration, whose values are known, and which regress from larger concentrations to smaller concentrations in Figure 1. The solutions in wells G1 and H1 are high and low controls with certain values. There are similar ratios between the photometric results obtained from the Elisa device and the intensity values obtained from the image analysis program of these 8 solutions. In the comparison given in Table 3, it was observed that if the sample concentration was high (positive), there was a deviation of approximately 5% - 10% between the results obtained by both methods.

Table 3. OD / Density Values of Calibrators and Controls

Calibrators & Controllers	OD Value	Density (number of pixels)
Cal 1	2,862	1,9819808
Cal 2	2,053	1,3737214
Cal 3	1,13	0,7802675
Cal 4	0,501	0,4261223
Cal 5	0,246	0,26986712
Cal 6	0,023	0,1555262
Cont. (H)	1,483	1
Cont. (L)	0,156	0,2440595

By using image processing techniques, it can be easily determined visually whether the results of the samples in the wells on the sample image are positive or negative. As can be seen from Figure 4, the colors of the samples with OD values greater than the positive control are located in the purple area in the electromagnetic spectrum. Using this method, it is observed that there is a visible relationship between the OD values and color intensities of the samples. Through the image processing program, it

was observed that there was a close relationship between the well density values in Table 3 and the OD values of the Elisa device. When we take the positive control with an OD value of 1.483 as a reference (density = 1); Density values of other wells are calculated automatically. In the results, we can say that the sample of which well has a density value above 1 is positive. Each of the image density values obtained through the program is actually a reference value. All devices with photometric reading system; They have at least one control with whom they can compare the test results. Therefore, this program we use determines the intensities of the pixel values it has obtained by reference to any well we specify. It was known that there were 5 patients (positive) in the sample results of the sample plate used in our study. It was observed that the same wells of the plate were positive with the density values obtained through the image analysis program. The costs of Elisa reader devices are quite high compared to the cost of the Image Processing Program we use. There is no maintenance or repair of the program, and when a problem occurs with the program, it takes a very short time to fix the problem. It is quite simple for responsible people working in the laboratory to install the program on the computer with their own abilities. Since the program works using image processing techniques only with the received image; In case the Elisa reader devices malfunction or patients cannot receive results during the maintenance process, we are considered very profitable in terms of time. In cases where the devices cannot work, it is very important in terms of both time and continuity that the samples are pipetted into microplates by the laboratory personnel and after the necessary procedures are carried out, the picture of the plate can be taken and the analysis process can be started immediately.

4. Conclusion and Suggestions

More meaningful and accurate results can be obtained provided that more appropriate conditions and more specific photographs are taken. Such 96-well plate images can be taken more clearly with devices called Gel Imaging Systems, which are generally used in Research and Development laboratories. Images of the samples can be taken much more clearly with the dark room, various filter options, Epi-UV, Trans-UV, Epi-white light and Trans-white light options, and advanced and fixed camera in these systems. Therefore, the images of the sample images to be analyzed must be clear; It means that the density values to be taken will be closer to the current OD values. Because of to developing mobile communication tools, it has become easier to transport our data and use it in desired places. With the use of image processing programs on mobile devices; Photographing the samples with mobile phones and analyzing them at the same time will provide serious benefits in terms of time and practicality. Using the program in a laboratory environment; It can be used as an alternative method in almost all systems that provide visual results depending on both color change and photometric measurement technique (DNA-RNA band analysis, bacterial medium colony counting, etc.).

Declaration of Author Contributions

The authors declare that they have contributed equally to the article. All authors declare that they have seen/read and approved the final version of the article ready for publication.

Declaration of Conflicts of Interest

All authors declare that there is no conflict of interest related to this article.

Acknowledgment

This study has been produced from the master thesis of Muhittin AKSOY.

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To Cite: Aksoy, M., Çambay, Z., Metin, S., 2024. Developing a Solution Density Analysis Program with Image Processing Technique. *MAS Journal of Applied Sciences*, 9(1): 117-126. DOI: http://dx.doi.org/10.5281/zenodo.10673417.