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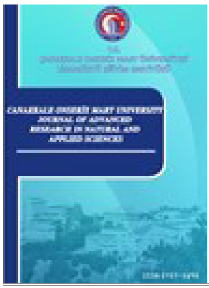

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Determination of Appropriate Thresholding Method in Segmenta-tion Stage in Detecting Breast Cancer Cells

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Abstract − As in all cancer types, the early detection of breast cancer is vital in terms of patients hold-ing on to life. Today, computer-aided image processing systems play an important role in the detection of diseases. Analyzing the imag-es with accurate image processing methods is very important for professionals to interpret the images and to devel-op the treatment methods for diseases appropriately. The images containing cancer cells (tumoroid) used in this study were obtained from the mini-Opto to-mography device that creates 3D images by reconstruction of 2D imag-es taken from different angles. It is an electronic, mechanical, and software-based device capable of 3D imaging of tumoroids up to 1 cm in diameter in size. Observing an entire tumor spheroid that has the size of several centi-meters in size in a single square image with a microscope is not possible, but with mini-Opto tomography it is possi-ble. In our study, a few layers of 3D images of the tumoroid produced by MCF-7 breast cancer cells obtained on the different days from the mini-Opto device were used. Image thresholding offers many advantages at the seg-mentation stage in order to distinguish the target objects. In this study, the determination of the most appropriate thresholding method for detecting the main tumor masses in the layered images was investigated. Moreover, the contours of the tumoroid were determined in the original images based on applying the outcomes of thresholding. While various thresholding methods have been applied on diverse images in the literature, we have applied a few thresholding methods to small tumors up to 2 mm in size. As a result of the qualitative assessment based on the results of the contour drawings on the thresholded images, the global thresholding and adaptive thresholding meth-ods gave the best results.

Keywords *− Breast cancer segmentation, thresholding methods, image processing, MCF-7 cancer cells, breast cancer cells, tumoroid.*

1. Introduction

In the world, women with breast cancer are deprived of early diagnosis and treatment of the disease because of many reasons such as social, economic, and geographic reasons, and these women pass away due to lack of intervention. When breast cancer is detected early depending on its type and with the right intervention at the right time, a significant increase in the life span of patients has been observed. There are different treatment methods for breast cancer in women, and choosing the best treatment method depends on the nature of the tumor, the cancer stage, and the preference of the patient (Waks & Winer, 2019)). In order to develop effective treatment methods, tumorous structures must be screened using various imaging meth-ods. Although X-ray imaging has been used for many years to diagnose the disease, many new methods have been developed up to date (Alarabeyyat & Alhanahnah, 2016). Another method of detecting breast cancer is that women regularly check their breast areas. The mammography method is an imaging method used to view cancer by sending low-dose X-rays to the breast and it is widely used (Kulkarni, Bhagyashree, & Udupi, 2010).

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The images used in our study are the images obtained from the mini-Opto tomography device, which is formed by blending the digital breast tomosynthesis which has the ability to view the breast organ in three dimensions and the microscope device that displays the microscopic cells in two dimensions. Mini-Opto tomography device is an electronic, mechanical and software-based program that creates a new three-dimensional image by reconstruction method from images taken from different angles in two dimensions (Polat, et al., 2019a).

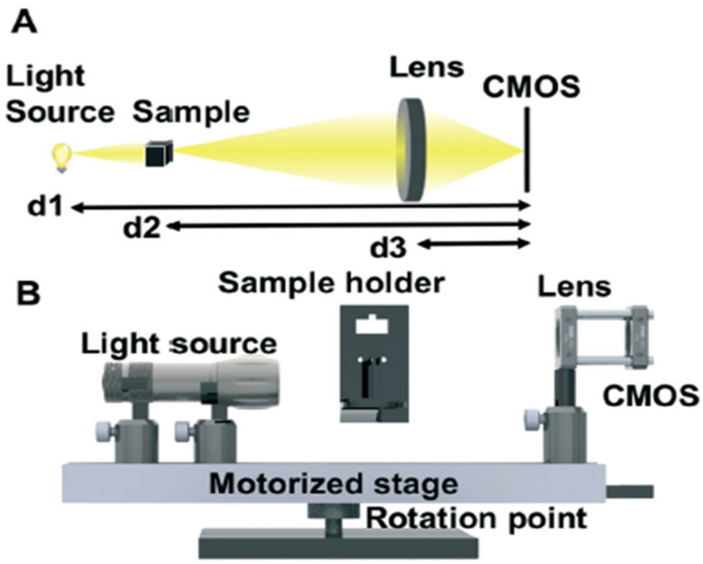

Figure 1. Basic structure of mini-Opto tomography device. A) Optical structure. B) Mechanical structure.

Figure 1 shows the basic structure of the mini-Opto tomography device. After the sample is placed in the holder, the light-camera system takes various images by moving at different angles around the sample to be imaged. These images go through a series of processes in the Matlab environment using (3D total variation (TV) regularized algebraic reconstruction technique (ART)) and finally, a new three-dimensional layered image is created (Polat, Matela, Dinler, Zhang, & Yildirim, 2019b). Since it is difficult to apply image processing techniques on to 3D images, these images must be converted into 2D layered images. Figure 2 shows the 2D images of 3 different days obtained from the 3D layered image.

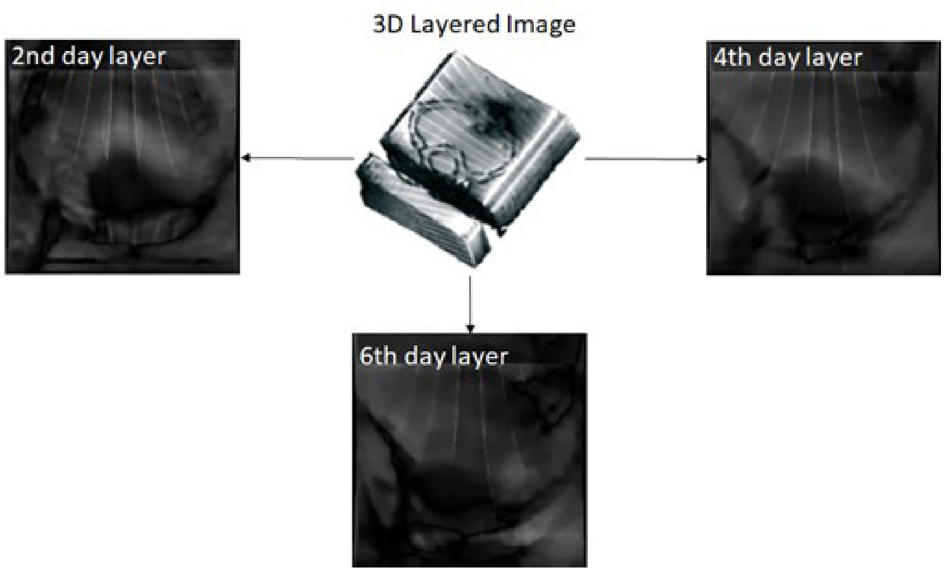

Figure 2. 2D layer images of the fifth day obtained for three different days from the 3D layered image.

There are many important studies in the literature for the thresholding process used in the segmentation step, which is an important step in breast cancer detection. Badawy et. al., applied the double thresholding method on mammogram images in breast cancer segmentation. It was concluded that the applied method not only made significant contributions to the detection of breast cancer in mammogram images but also had significant advantages in the processing time and storage area (Badawy, Hefnawy, Zidan, & GadAllah, 2017). Fuzzy entropy with a level set (FELs) thresholding method using on various cancer images is promising in medical image segmentation and can be used to classify cancer types according to the clinical diagnosis method (Maolood, Al-Salhi, & Lu, 2018). Kumar et., al. concluded that multilevel thresholding is more efficient than classical thresholding methods, according to the results obtained from analysis of metrics in the segmentation of digital mammogram images (Kumar, Kumar, Bajaj, & Singh, 2018). Jian et al. proposed the color thresholding method for image segmentation of blood cells. As a result of the performance analysis of the color thresholding method, the qualitative and quantitative evaluation of this method was made and as a result, they concluded that the method was effective in the segmentation of blood cells (Jian, Nazahah, Yusoff, & Shakir, 2019).

2. Materials and Methods

The following algorithm was used to separate the main tumor masses from the image background in the layered images obtained from the mini-Opto tomography device.

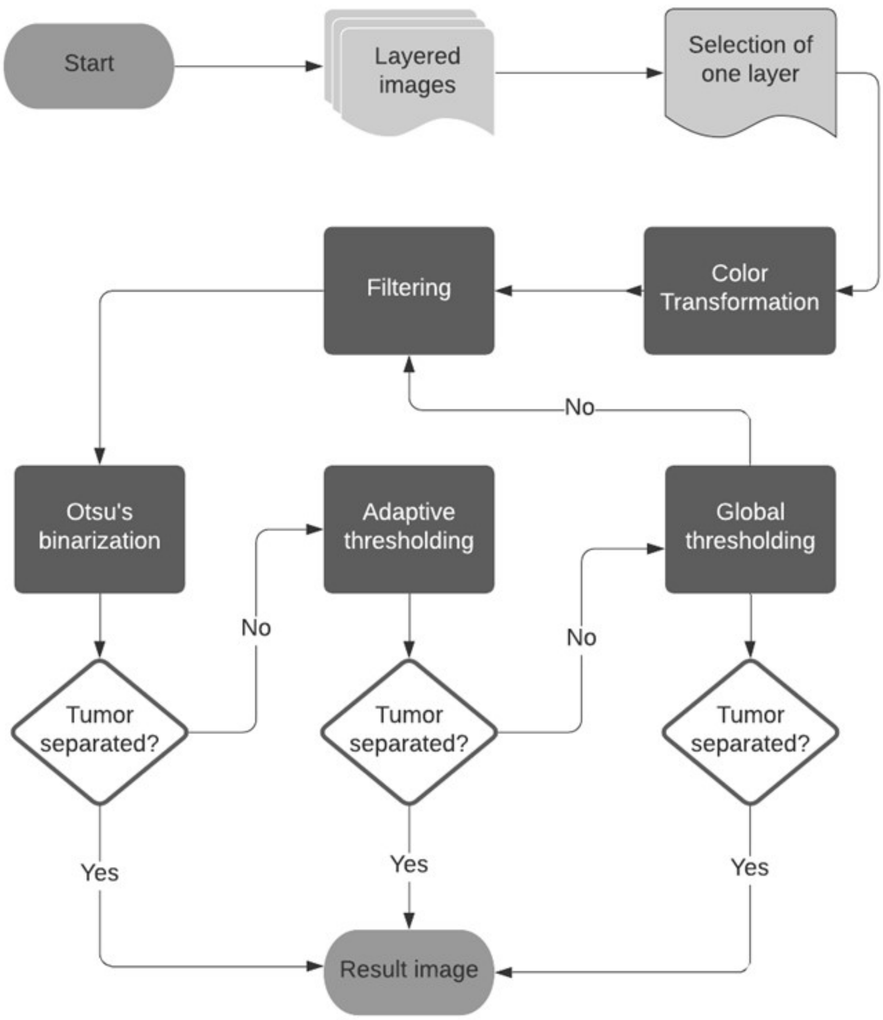

Figure 3. The general representation of the image segmentation process and the decision of the appropriate thresholding method.

Looking at Figure 3, after the layered images are obtained from the mini-Opto tomography device, the images are pre-processed in order to make these images ready for the segmentation step. In the pre-processing process, images are converted into color tones between 0-255, that is, color tones between black and white, which have less intensity information than color images. Another important step in pre-processing is the filtering process, which enables the images to be further developed. In this process, unwanted objects such as unnecessary details and noises on the images are eliminated and a more advanced image is obtained. After the pre-processing step, the thresholding process, which is the basis of the work, is performed in the segmentation step. In the thresholding step, the pixels in the image are divided into two different groups, namely 0 and 1, so we are one step closer to the main tumor mass. In order to distinguish the main tumor mass in the image from the image background, an appropriate thresholding method should be chosen. In our study, global thresholding, adaptive thresholding and Otsu's binarization methods were applied, respectively, for images that passed through the preprocessing stage.

The global thresholding method is the simplest thresholding method and since the difference between the object and the background in the image is significant, a single threshold value is used to separate the object from the background (Senthilkumaran & Vaithegi, 2016). In the global thresholding operation, the value is calculated by the following formula.

$$g(x,y) = \begin{cases} 1, & src(x,y) > T(x,y) \\ 0, & src(x,y) \le T(x,y) \end{cases} \tag{2.1}$$

In 2.1, value is the pixel value of the source image, is the threshold value determined to separate the object in the image from the image background, and is the binary image obtained as a result of the thresholding process. When the pixel value with coordinate is greater than the threshold value, the result is 1 and a value of 1 represents the object in the image. Otherwise, the result is 0 and a value of 0 represents the background. In our study, the main tumor structure in the cancerous image represents the object and other parts in the image represent the background.

In another thresholding method, the adaptive thresholding method, when the objects in the image have different light intensities, a single threshold value will not give accurate results in image segmentation (Sujji, Lakshmi, & Jiji, 2013). Therefore, different threshold values are used in different parts of the image. In other words, the image is made binary by using more than one threshold value instead of a single threshold value. In the adaptive thresholding method, there are two methods that determine how to calculate the threshold value, mean adaptive and gaussian adaptive. In the mean average weighted adaptive thresholding method, the threshold value is the average of the areas of neighboring pixels, while in the gaussian weighted adaptive thresholding method, the threshold value is the weighted sum of the neighborhood values where the weights are a Gaussian window (Mapayi, Viriri, & Tapamo, 2015; Mordvintsev & Abid, 2017).

Normally, a threshold value is determined to convert a gray image to a binary form, and image pixels are grouped into object and background to this threshold value. However, in some cases, fixed threshold values may not give accurate results on all images. It is based on the approach of minimizing the weighted sum of the object and the background pixels in the image in order to create an optimum threshold value in the Otsu method, which is based on threshold selection according to statistical criteria (Al-Tarawneh, 2012). Otsu method has a value that will maximize the variance between these two classes by accepting that there are two separate classes on the image (Liu & Yu, 2009). The interclass variance is calculated by the following formula.

$$\sigma_s = \sigma - \sigma_b = w_1(t)w_2(t)(\mu_1(t) - \mu_2(t))^2 \tag{2.2}$$

Here, variables are class intensities and variables are weighted class averages. Weighted class averages are calculated as follows.

$$\mu_1(t) = \sum_{i=0}^{t} P_r\{i\}\ H\{i\} \tag{2.3}$$

$$\mu_2(t) = \sum_{i=t+1}^{255} P_r\{i\}\ H\{i\} \tag{2.4}$$

In Equations 2.3 and 2.4, gives the histogram value belonging to the th color level, and the value, which gives the maximum variance value by calculating the variance for each value from 0 to 255, is accepted as the threshold value. The results obtained by applying the global, adaptive and Otsu's thresholding methods, which are mentioned in Chapter 2, on cancer images of the second, fourth and sixth days are discussed in the third section.

Computer-aided image processing systems are used to detect suspicious lesions on cancerous images, and these systems can produce output about the type of cancer in addition to detecting suspicious areas. Thus, radiologists make detailed analyzes using these outputs and interpret the images (Yassin, Omran, El Houby, & Allam, 2018). In our study, a qualitative analysis of the thresholding methods that can best distinguish the main tumor masses from the image background in the images was performed. Python programming language and OpenCV library were used as computer-aided diagnostic systems on the images.

Our python code consists of three main steps. The first step is to upload the images. In the second step, which is the preprocessing, it was aimed to enhance the images and make them ready for the segmentation step. In the third step that is the segmentation part, various thresholding processes were applied to separate the main mass from the background of the image. The pseudocode of the process is presented below.

```
Installation OpenCv library packages
        - Import Numerical Python package
        - Import Image read package
        - Import image processing functions
Step 1. Uploading raw images
        >> for i = each image
            importing image [i]
        >>end
Step 2.  Pre-processing
        Step 2.1. Convert colored image to gray type image
        Step 2.2. Apply filters to the converted image
            >> for k = each filter
                >> for i = each image
                    apply filter[k] filter to gray image[i]
                >>end
            >>end

Step 3. Segmentation
        Step 3.1. Apply thresholding methods to images
            >> for m = each thresholding methods
                >> for n = each filtered image
apply thresholding[m] to filtered image[n]
                    find counter image[n]
                    draw counter image[n]
                    Find the main tumor mass contour and delete the other contours in the image[n]
                >>end
            >>end
```

3. Results and Discussion

Figure 4, 5 and 6 show the results obtained when different thresholding methods are applied to the fifth layer images belonging to the second, fourth, and sixth days in the segmentation stage. On day 2, the main tumor mass was separated from the image background as a result of the global thresholding method, one of the thresholding methods, and this method gave the best result. Selecting a single threshold value on the raw image was successful in separating the main tumor mass from the image background.

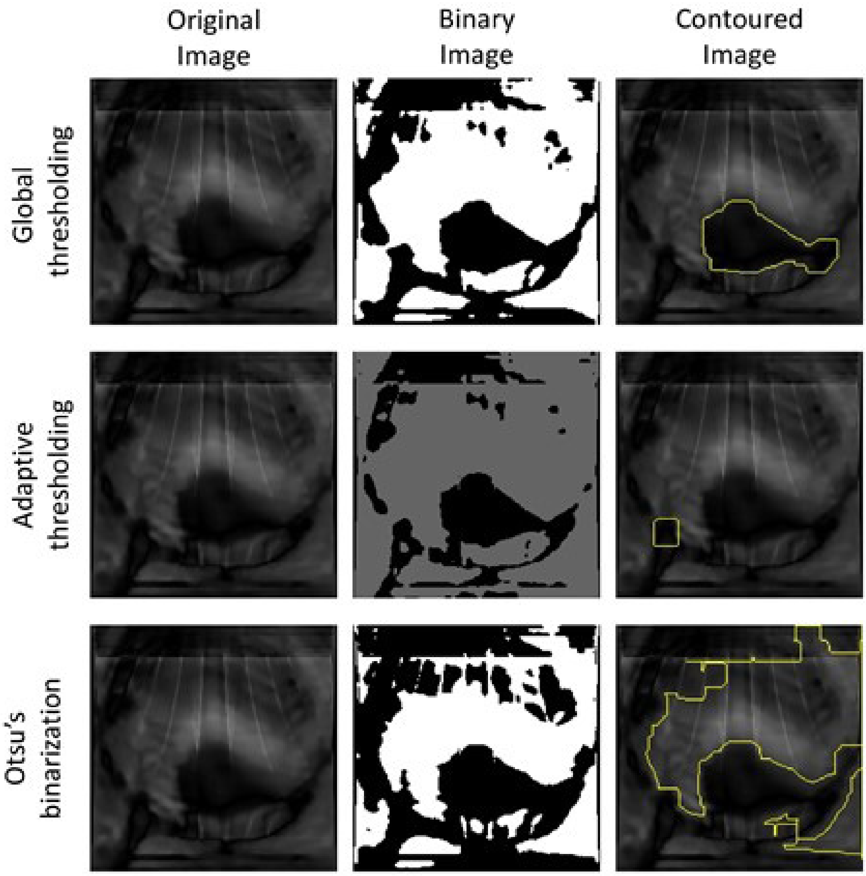

Figure 4. The result images obtained when different thresholding methods are applied to the fifth layer on the second day.

Figure 5 shows the result images when 3 different thresholding methods are applied to the fourth-day image. The global thresholding method detected a region close to the main tumor mass. But, as can be seen from the figure, the adaptive thresholding method gave the most successful result in separating the main tumor mass from the image background. Because the main tumor mass has different light intensity, more than one threshold value was determined rather than a single threshold value to separate the main tumor mass from the image background.

Looking at Figure 6, the global thresholding and adaptive thresholding methods gave the most successful results in revealing the main tumor mass on the sixth day image. Since the main tumor structure has different light intensity, the adaptive thresholding method gave more successful results than the global thresholding method.

Since the pixel distributions between the object and the background in the second, fourth and sixth day images are very different from each other, the correct threshold value could not be calculated exactly. Therefore, the Otsu's binarization method did not give successful results on the images.

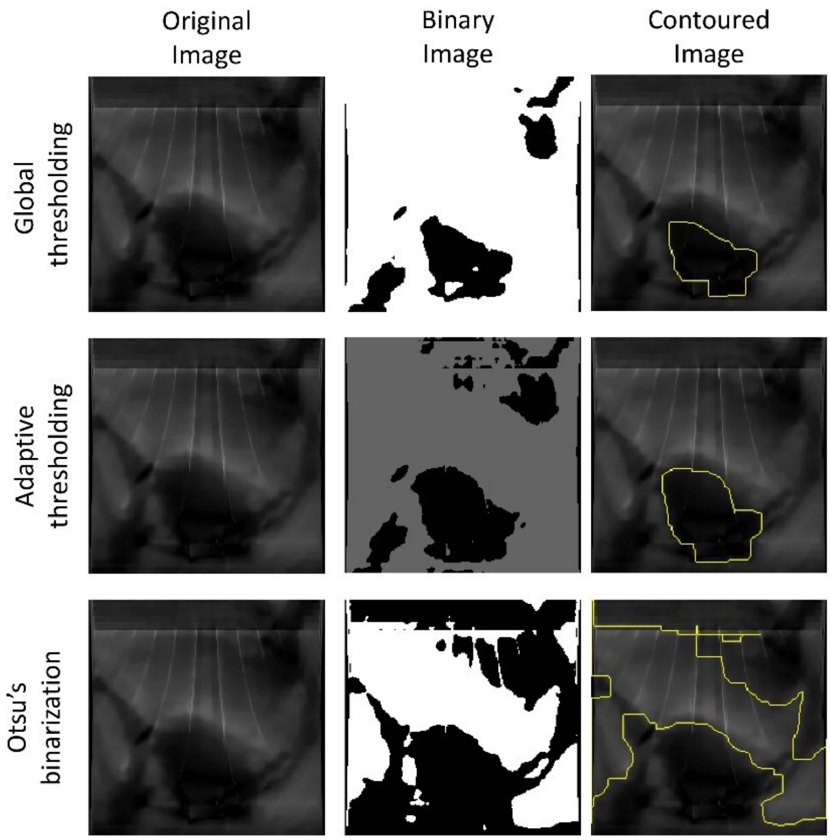

Figure 5. The result images obtained when different thresholding methods are applied to the fifth layer on the fourth day.

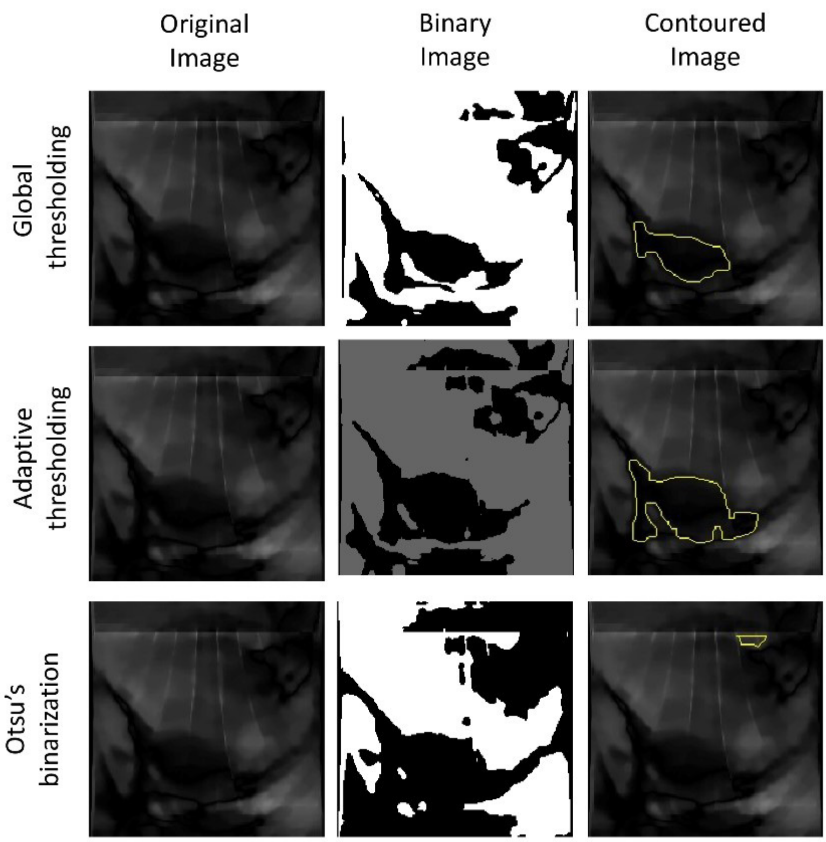

Figure 6. The result images obtained when different thresholding methods are applied to the fifth layer on the sixth day.

When our study is compared with similar studies in the literature, it was seen that there are some similarities and differences. In a study which conducted by Badawy et. al., a double thresholding has been proposed for the segmentation of mammogram images (Badawy, et. al., 2017). Because the detection of scattered details in mammogram structures was necessary. However, we used the global thresholding method, which consists of a single threshold value, since there was a significant difference between the color tone of the main tumor structure and the ones of the image background in our image on the 5th day. Another study, the reference (Kumar, et. al., 2018), claimed that multi-level thresholding method yields better results than the classical thresholding method, as more density is used to represent objects in a breast cancer image. Unlike in our study, the global thresholding method which is a classical thresholding method applied to the second-day image gave a more successful result than the multi-level thresholding method. The biggest reason for this situation is that although the color levels are similar when the image is examined in its entirety, the pixel density of the main tumor mass is significantly different from the rest of the image. In a study conducted by Joseph et. al., while K-means clustering which is an unsupervised and clustering algorithm followed by morphological filtering has been proposed to detect tumor structure in brain MRI images (Joseph, Singh, & Manikandan, 2014). In our study, we were able to detect the tumor structure without the need for further operation by using the thresholding method at the segmentation stage.

4. Conclusion

Breast cancer is the second most common type of cancer in the world and the first among cancer types seen in women. Therefore, early detection is vital for the patient's life. There are various medical imaging methods for early detection of diverse types of cancer. In our study, the mini-Opto tomography device was used to analyze breast cancer cells that build tumors up to 2 mm in size. Image processing methods were used on the two-dimensional images of the tumor spheroid produced in the laboratory, obtained with the device. We focused on some thresholding methods to separate the main tumor structures in the images from the image background. Among these thresholding methods we used in the study, the global thresholding and the adaptive thresholding methods gave the best results. The results showed that the thresholding method alone without a requirement of additional operation could be sufficient to separate the main structures in an image from the background of that image. The biggest limitation of the study is the low resolution of the images obtained from the mini-Opto tomography device. Considering this limitation, especially in tumor-background border crossing regions, the cells that cover border pixels of the main tumor structure could not be distinguished clearly. Therefore, in the future, by strengthening the technological infrastructure of the mini-Opto tomography platform, high-resolution images would be obtained and the image processing techniques to be applied to these images would yield results that are more superior. In future studies, it is aimed to diversify more thresholding methods used in the segmentation step of image processing methods to detect cancerous cells in images. Moreover, thresholding methods could be combined with various morphological operations to analyze the developments of the detected tumors in their own habitat.

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Author Contributions

Adem Polat: Conceived the original concept, designed the methodology and the experiments, and wrote the manuscript.

Cihat Ediz Akbaba: Applied the methodology, analyzed the simulations, and wrote the manuscript.

Conflicts of Interest

The authors declare no conflict of interest.

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