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Enhancement of DNA Microarray Images Using Mathematical Morphological Image Processing

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ABSTRACT

DNA microarray images contain spots that represent the gene expression showing the condition of normal and cancer samples. As there are numerous spots on DNA microarray images, image processing is one of the methods to enhance and analyse the image. Some contain noises or contaminations from different process stages, which may lead to a false conclusion based on the gene expression information. The mathematical morphology is proposed to enhance the microarray image and analyse the noise removal on the image. This follows an experiment in which the erosion, dilation, opening, closing, white top-hat (WTH) and black top-hat (BTH) operations were applied on a DNA microarray image and the results for each operational process were analysed. The noises were completely removed by the erosion operation and the images were completely enhanced. Based on the results, mathematical morphological image processing enhanced the microarray image and the noises were completely removed by the erosion operation.

Keywords: DNA microarray image; mathematical morphology; image enhancement

INTRODUCTION

Microarray technology is widely known for allowing scientists to analyse the gene expression. Furthermore, microarrays make it easier to compare normal with cancerous cells. For this study, deoxyribonucleic acid (DNA) microarray containing microscopic DNA spots were deposited on the surface of a glass slide. Firstly, two samples of cDNAs (normal and cancerous cells) were labelled with different fluorescent dyes (Cy3 and Cy5) (Belean et al., 2015; Helmy & El-taweel, 2013). Then, both samples were hybridized on the same glass slide. Later, the glass slide was scanned using a green and red laser after the hybridization process was completed. After that, the composite image was produced and the intensities of each spot were analysed. The green colour appeared when the sample that was labelled using Cy3 was in abundance. Otherwise, the red colour would have appeared because the sample that was labelled using Cy5 was in abundance. If the two samples were in equal abundance, the yellow colour would have appeared; if neither sample was present, it would have appeared as a black colour (Qin et al., 2005). Therefore, the gene expression of the DNA microarray images can be monitored. The DNA microarray images may contain noises and contaminations during the scanning process or during previous process stages (Qin et al., 2005; Wang et al., 2013). These problems can affect the whole microarray image process. Thus, image processing is proposed as a solution in order to minimize or eliminate the causes.

Microarray image processing contains the following three stages: (1) gridding, which is a process of segmenting the microarray image into partitions, with each partition having only one spot; (2) segmentation, which is a process of differentiating between the foreground and background features; and (3) intensity extraction, which is a process of calculating the intensity that is available on the image (Harikiran et al., 2012; J et al., 2011). The noises on the microarray image cause a major problem during the entirety of image processing. Some of them may generate wrong information about the gene expression. Besides, missing spots on the microarray image may also appear. Thus, the process of spot recognition is a difficult procedure as the microarray image consists of noises that interrupt the image acquisition (J et al., 2013). Therefore, it is more convenient if the noise is removed or minimized at an earlier stage. Image enhancement is an important method because it can recover several useful details on the image from the noise environment (Bai et al., 2012).

In this paper, mathematical morphological image processing was performed on a DNA microarray image, with each operation in mathematical morphology analysed. The operations were programmed using the MATLAB software; the output image for each operation is discussed. Literature review section discusses several applications that have used mathematical morphology in image processing. Methodology section explains the methodology that was used during the study. In result and discussion section, all the experimental results are discussed based on the mathematical morphological operations. Lastly, the conclusions of the work are written on the last section.

LITERATURE REVIEW

Mehta et al. (2015) proposes the combination of pre-processing morphology and entropy calculation to enhance the ultrasound images of the gall bladder. The ultrasound images are first converted into greyscale and then into a black and white image using a threshold filter. The threshold value of this work is set at 0.18. By converting the image into a black and white image, the image itself is able to distinguish between the foreground and background features. The erosion and dilation operations are used to improve the clarity of the image, in which the erosion shrinks the foreground features, while the dilation enlarges the foreground features. Based on the result, the ultrasound image clearly shows the location of the gallstone, while the image has improved significantly compared to the input image.

In paper Mittal & Dubey (2013), the author proposes using morphological image processing for early detection of rheumatoid arthritis (RA). This disease is commonly caused by inflammation in the joints, fingers and knees. The erosion process shrinks the foreground features of the image, while the dilation process enlarges the foreground features of the image. Both processes use the same structuring element (SE) as a probe. Based on the result, morphological image processing provides a better understanding of the ultrasound image and can improve the monitoring of RA.

Yuan & Li (2015) demonstrates a switching morphological and median (SMM) filter as a proposed for noise removal. Morphological operations, such as erosion, dilation, opening and closing, are applied in order to eliminate the noise. The noise pixel can be estimated by combining the output of the conditional morphological filter with that of the improved median filter. The proposed method provides higher peak signal-to-noise ratio (PSNR) values compared to boundary discriminative noise detection (BDND), fast switching median (FSM), convolution noise detection-based switching median (CNDSM), opening-closing sequence (OCS), efficient edge-preserving (EEP) and noise adaptive fuzzy switching median (NAFSM) filters. Based on the result, the proposed method removes the noise effectively and the details of the image are well preserved.

Rong-yu et al. (2012) concerns the observation of space objects using a full-frame transfer CCD camera to detect and locate stars and moving objects in space. During the observation, the CCD images that are produced contain a smearing effect because the camera shutter is often removed. The effect degrades the image quality and increases the difficulty of object recognition. The author proposes mathematical morphology in order to eliminate the smearing effect as well as improve the detection rates and position accuracies of stars and moving objects. The dilation operation filters the maximum value depending on the structural body. The erosion operation filters the minimum value depending on the structural body. The opening operations eliminate the bright details that reduce the size of a structural body. The closing operation eliminates the dark details that reduce the size of an SE. This paper uses TopHat and Spread TopHat methods to remove the long-strip signals in the CCD image. The structural body plays an important role in morphological results, such that different structural bodies give different results. Based on the result, mathematical morphology improves the detection rate and position accuracies of stars and moving objects by eliminating the smearing effect.

Zhang et al. (2011) proposes using an improved morphological edge detection method for an edge detection operator and an iterative thresholding method for a better thresholding value in order to identify foreign fibres in cotton products. Dilation and erosion algorithms are used in the edge detection operator. Each colour (R, G and B) is then taken into account in the image, while the improved morphological edge detection consists of the edge detection for each colour, which is called the edge intensity value; if the value of it is greater than the given threshold, it is considered as edge pixels from the image. Therefore, the iterative method is a method to select the threshold value automatically because different situations lead to different light intensities. Based on the result, the proposed methods provide a better accuracy for the segmentation of foreign fibres and improve the processing time compared to conventional methods.

TABLE 1 Comparison of different applications.

Method	(Mehta et al., 2015)	(Mittal & Dubey, 2013)	(Yuan & Li, 2015)	(Rong-yu et al., 2012)	(Zhang et al., 2011)
Application	Medical	Medical	Case study	Astronomy	Agriculture
Type of image	Colour	Greyscale	Greyscale	Greyscale	Colour
Threshold	Yes	No	No	No	Yes
Accuracy	N/A	N/A	N/A	N/A	N/A
Complexity	Low	Low	Normal	Normal	Normal
Special feature	N/A	N/A	SMM	TopHat and Spread TopHat	Morphological edge detection and iterative thresholding

Mathematical morphological image processing operations help to enhance the features on the image. Different applications will use different operations of mathematical morphology. Table I shows the comparison between different applications that apply mathematical morphological operation, which will be discussed later. Yuan & Li (2015) uses the SMM filter to determine the noise pixels in relation to the removal process. TopHat and Spread TopHat transforms in method (Rong-yu et al., 2012) are discussed, in which the smearing effect may be eliminated by choosing suitable SEs. Zhang et al. (2011) uses a combination of morphological edge detection and iterative thresholding to improve the segmentation process in order to identify foreign fibres in cotton. From the five applications, it can be seen that mathematical morphological operations may enhance the image and preserve image information well. In the next section, the experiment undertaken in this work is presented.

METHODOLOGY

This section discusses mathematical morphological image processing of the real microarray image (‘scanner-images.jpg (2200×7300)’, n.d.). The mathematical morphological process is programmed using the MATLAB software. Fig. 1(a) shows a part image with a size of 441×431 pixels, compared with the real microarray image of 2200×7300 pixels, which is used as the input image for this work. Fig. 1 (b) shows the SE that is used as a probe for mathematical morphology. An SE of a disk shape, with a radius of four pixels, is chosen as the input image to be used, containing DNA spots, which are generally in circle shape. Therefore, the disk shape in structuring is used to produce similarity with the information interest on the input image. The spots on the input image provide the important information, so that the SE of mathematical morphology may enhance the spots area that forms the background. The size of the SE depends on the spot size. In this work, the radius of four pixels is chosen because the average size (diameter) of the spots on the input image is 8~9 pixels.

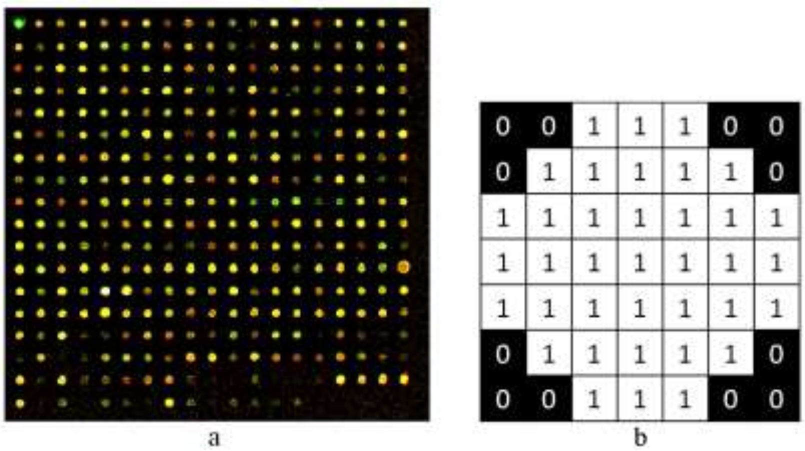

Fig. 1: (a) Input image of a 441×431 pixels region from a real microarray image of 2200×7300 pixels; (b) the SE of a disk shape with a radius of four pixels.

Mathematical morphological image processing

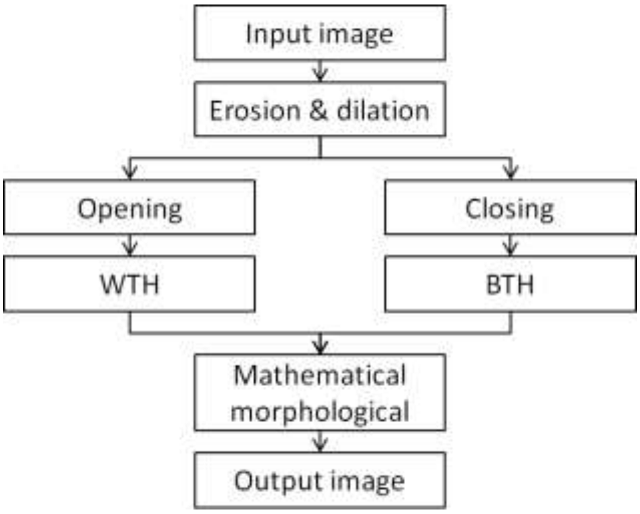

Fig. 2: The flowchart of mathematical morphological image processing.

Fig. 2 shows the flowchart of mathematical morphological image processing, of which the basic process is erosion and dilation. Erosion is a process of removing the foreground features on the image. Dilation is a process of adding the foreground features on the image. Both the erosion and dilation processes depend on the SE. Opening is a process to remove the foreground objects that are smaller than the SE. Closing is a process of removing the background objects that reduce the SE. Both opening and closing control the uses of erosion and dilation to enhance the foreground and background features. During the opening, the input image (IM) will undergo erosion first, followed by dilation. Meanwhile, in dilation, the IM will undergo dilation first, followed by erosion. Next, the major process of mathematical morphology involves the white top-hat (WTH) and the black top-hat (BTH). The WTH enhances the foreground features on the image in line with the different results obtained between the IM and the opening operation. Meanwhile, the BTH enhances the background features on the image with the different results obtained between the closing operation and the IM. Lastly, mathematical morphology is the result of the IM added to the WTH, then subtracted from the BTH. The opening, closing, WTH, BTH and overall operations are defined as follows (Li et al. 2015):

$$Opening = IM \ominus SE \oplus SE \tag{1}$$

$$Closing = IM \oplus SE \ominus SE \tag{2}$$

$$WTH = IM - Opening \tag{3}$$

$$BTH = Closing - IM \tag{4}$$

$$Mathematical\ morphological = IM + WTH - BTH \tag{5}$$

where $\ominus$ and $\oplus$ respectively denote the erosion process and the dilation process.

RESULTS AND DISCUSSION

In the previous section, the mathematical morphological algorithm was presented. The algorithm was run on the input image as shown in Fig. 1(a) using the SE shown in Fig. 1 (b). In this section, the experimental result for each process in the mathematical morphological operation will be presented. All the workings are performed using the MATLAB software running on the Windows operating system. Firstly, the input image will convert into the greyscale image, as shown in Fig. 3 (a). The image result for each operation is zoomed in with 161×166 pixels, which contain 7×7 spots for ease of analysing and understanding each operation, as shown in Fig. 3 (b).

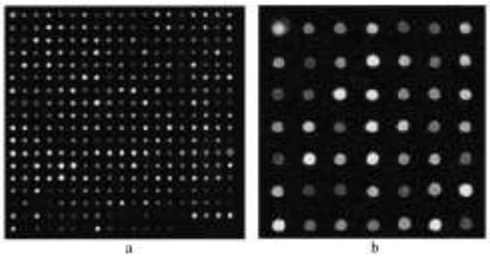

Fig. 3: (a) The greyscale image of the input image; (b) the rescale input image consists of 7×7 spots.

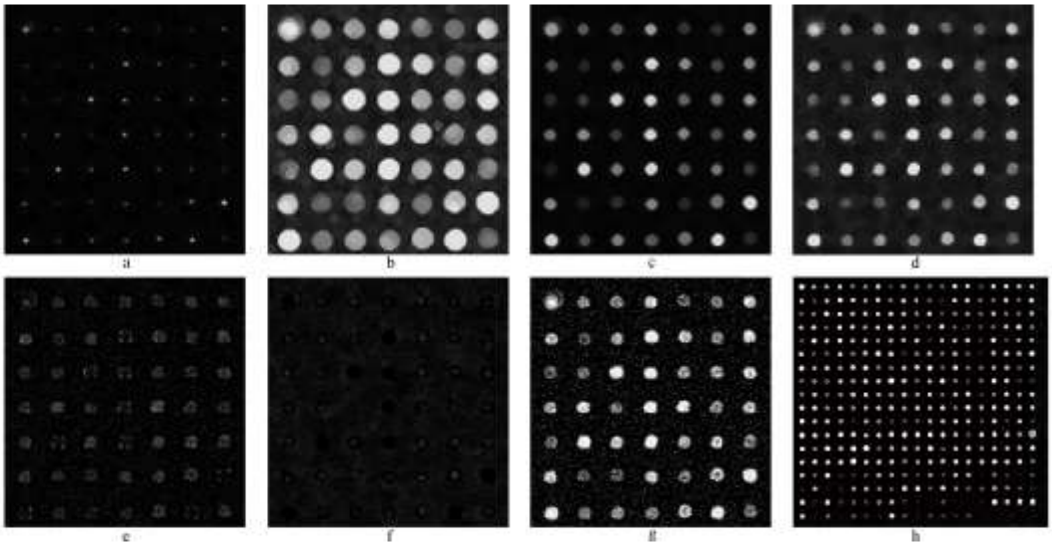

Fig. 4: (a) The result of the erosion operation; (b) the result of the dilation operation; (c) the result of the opening operation; (d) the result of the closing operation; (e) the result of the WTH operation; (f) the result of the BTH operation; (g) the result of the mathematical morphological operation; (h) the final result of the full scale of the input image.

Opening, closing, Erosion and dilation operations

The erosion operation shrinks the foreground features depending on the SE, as shown in Fig. 4 (a). Based on the result, the spots (foreground features) on the image shrink due to the erosion operation. The shrinking causes a decrease in the foreground area compared to the input image. At this point, the noises that are smaller than the SE are completely removed. Besides, the spots that are smaller than the SE can also be removed, so selecting the appropriate size of the SE is important for this operation. The dilation operation enlarges the foreground features depending on the SE, as shown in Fig. 4 (b). Based on the result, the spots (foreground features) on the image become larger due to the dilation operation. The enlargement causes a decrease in the background area compared to the input image. At this point, the noises are also enlarged with the spots. The spots might combine with other foreground features if the size of the SE is too large.

Next are the opening and closing operations, in which the opening is a process to remove the foreground objects that are smaller than the SE while the closing is a process of removing the background objects that are smaller than the SE. From the result in Fig. 4 (c), it shows that the opening operation removes the foreground object that is smaller than the SE compared to the input image. Based on the result, the opening operation is generally run as the dilation operation on the image result from the erosion operation in Fig. 4 (a). From the erosion operation, the foreground object that is smaller than the SE is already removed. Thus, the opening operation is the result of the enlargement of the foreground object that remains after the erosion operation. The opening operation follows the mathematical theoretical state, as given at (1).

From the result in Fig. 4 (d), it shows that the closing operation removes the background object that is smaller than the SE compared to the input image. Based on the result, the closing operation is generally run alongside the erosion operation on the image resulting from the dilation operation in Fig. 4 (b). From the dilation operation, the foreground features are enlarged, with some of them combining with other spots or noises. Thus, the closing operation is the result of shrinking the foreground object on the image resulting from the dilation operation. The closing operation follows the mathematical theoretical state, as given at (2).

Mathematical morphological, WTH and BTH operations

Next, the major operations in mathematical morphology take place, which concern the WTH and the BTH. The WTH is the difference between the input image and the opening operation result, as stated in (3). Based on the result in Fig. 4 (e), the foreground features that remain are the exceeded pixels in the foreground on the input image compared to the image result from the opening operation. The BTH is the difference between the closing operation result and the input image, as stated in (4). Based on the result in Fig. 4 (f), the foreground features that remain are the exceeded pixels in the foreground on the image result from the closing operation compared to the input image. Finally, from the result of the WTH and the BTH, the enhancement of the image can be produced. The mathematical morphological operation is a process where the input image is added to the image result from the WTH operation and then subtracted with the image result from the BTH operation, as stated in (5). Based on the result in Fig. 4 (g), the image becomes sharper and clearer compared to the input image. Fig. 4 (h) shows the final result of mathematical morphology on the full scale input image (Fig. 3 (a)).

CONCLUSION

In this paper, the mathematical morphological image processing shows a better enhancement of the microarray image. First, the erosion and dilation are fundamental to the mathematical morphology. Next, the opening and closing operations enhance the foreground features and background features, respectively. Lastly, the mathematical morphological operation produces the enhancement image by adding the input image and the WTH image, and then subtracting the BTH image. Based on the result, the mathematical morphological image processing better enhances the microarray image compared to the input image. Besides, during the erosion operation, the noises are completely removed.

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