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A NOVEL APPROACH FOR MICROARRAY SPOT SEGMENTATION & DETECTION USING FOUR SHAPED MATHEMATICAL MORPHOLOGY

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Abstract- Microarray is new techniques to investigate the expression levels of thousands of genes simultaneously. Image analysis is an important aspect of microarray experiments, one, which can have a potentially large impact on subsequent analysis such as clustering or the identification of differentially expressed genes. Microarray image spot position & size determination is a very important step in the analysis of microarray image because it is the first part we need to do for the analysis. By comparing gene expression in normal and abnormal cells, microarrays may be used to identify genes, which are involved in particular diseases. These genes may then be targeted by therapeutic drug. Making this part automated and fast is also important. So we consider this as indeed problem of correct spot detection and automatic counting. Overcome it by using flat structuring element (disk, diamond, square and octagon shaped) morphological segmentation technique, which gives satisfied results. And also we compare the original & resultant image results on the basis of different image quality measure.
Keywords- Microarray, flat structuring element, morphology, segmentation, spot detection.

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Introduction

Microarray images contain large number of spots need to be analyzed in a very fast automated way and this analysis is very important in discovering the cause of diseases [1,2,3]. To develop Segmentation technique for Microarray images, to cater the need of Microarray imaging to solve the problem specified.

The processing of the microarray images can be separated into three sequential steps namely,

- Griding
- Segmentation &
- Intensity extraction

The Analysis of microarray gene expression data involves many steps, the initial step consist of extracting the gene expression data from the microarray image and include spot localization or gridding [4,5,15], foreground and background separation, intensity extraction, clustering [17], Grid Alignment, Spot quality assessment, Quantification, Normalization, Identification, Data Mining [8,14,19]. These stages are quite important, since accuracy of the resulting data is essential in posterior analysis [6]. The DNA sequences are spotted on the slides. After image processing, gene expression data can be obtained for further analysis. Such as gene clustering and identification [7], segmentation- fixed circle, adaptive circle, adaptive shape, histogram and Seed Region Growing, addressing, information extraction, adaptive ellipse method [4,5,7,8]. However, one of the major challenges of this approach is the image-processing phase [14]. The purpose of this step is to extract each spotted DNA sequence as well as to obtain background estimates and image quality measures. DNA microarray can also be used to characterize the cellular differences between different responses between different tissue types, such as between normal cells and cancer cells and between cancers with different responses to treatment, or between control cells and cells treated with a particular drug. [7] Material consisted of 80 different Microarray images are download from publicly available database e.g. Microarray Genome Imaging & clustering Tool (MAGIC) website [22] and others sites. Motivation from this problem, in this paper we have applied morphological segmentation technique to determine these spots and got satisfied result as well as compare to disk, diamond, square shaped structuring element. Octagon shaped morphology is used and calculate the statistical parameters of original and resultant image our results are visibly and statistically more good. The over-segmentation problem is also removed.

Microarray Image Segmentation Technologies

Segmentation and background correction methods are used for extraction of information from microarray images. In some cases background adjustment can substantially reduce the precision that is increase the variability of low intensity spot values [10]. The background correction method uses an image analysis technique. In existing survey various techniques is found [4,5,7,14,17]. Out of the three steps of microarray image processing, Segmentation allows the classification of pixels as foreground (i.e. as corresponding to a spot of interest) or as background. Existing segmentation methods for microarray images can be categorized into five groups, according to geometry of the spots they produce,

1. Fixed circle segmentation,
2. Adaptive circle segmentation,
3. Adaptive shape segmentation,
4. Histogram segmentation,
5. Adaptive ellipse method.

But every method or technique have its own advantages and disadvantages, as mentioned in recent work fixed circle segmentation is easy to implement but a fixed diameter segmentation is clearly not satisfactory for all spots this technique is implemented by M. B. Esien software name is ScanAlyze [4]. In adaptive circle segmentation circles diameter is estimated separately for each spot but this can be time consuming since each array contain thousand of spots this technique is implemented by M. B. Esien software name is ScanAlyze, GenePix, ScanArray Express, Emagene & Dapple [14,17,18]. Where as in adaptive shape segmentation two methods are used watershed and seed region growing [17]. Both watershed & SRG requires the specification of starting point of segmentation procedures using these methods can be the selection of the number & location of the seed points. Microarray images are well suited to such techniques. But in histogram segmentation, each spot foreground and background intensity estimates are determined on the basis of histogram of pixel values or intensity values. [4] These methods do not use any local spatial information and these are simple methods. X. H. Wang et al. discusses an image enhancing technique by denoising using stationary wavelet transform. This technique can overall eliminate the random noise to improve the quality of gene expression data [20]. The main disadvantage of these methods is that quantitation is unstable when a large target mask is set to compensate for spot size. Some existing methods include manual processing of DNA spot images using a generic image-processing tool, such as NIH image. [21] But no one method produces the increase in number of spots counting is automated, which are present in the image after segmentation of background and foreground. To overcome this we have set the objective for this paper and overcome this problem up to certain level by using morphological transforms with some morphological operations.

Methodology

Structuring element (SE), of the type specified by shape. Depending on shape, structuring element can take additional parameters. There are two types of structuring element [11],

i. Flat Structuring Element,
ii. Non-flat Structuring Element. We use flat structuring element.

Morphological Operations

Morphology technique is used to operate on images via different operations; few of them are given here.

Closing and Opening

By iteratively applying erosion and dilation, one can eliminate image details, smaller than the structuring element, without affecting its global geometric features.

Visually, closing is smoothes the contours by filling in narrow gulfs and eliminating small holes. The closing operation is simply a dilation operation followed by an erosion operation. The gray scale closing of A by B, denoted by $(A \bullet B)$, is defined as,

$$(A \bullet B) = (A \oplus B)\Theta B.......(1)$$

On the other hand, opening smoothes contours by breaking narrow isthmuses and suppressing small islands. The opening operation is simply an erosion operation followed by a dilation operation. The gray scale opening of A by B, denoted by $(A \circ B)$, is defined as,

$$(A \circ B) = (A \Theta B) \oplus B.......(2)$$

In this section we briefly introduce the basic principles, definitions and notations used in mathematical morphology [10,12,16].

When we perform the morphological operation like dilation and erosion the structuring element plays an important role, which is a powerful tool to extract information from images [5].

Structuring element is a small grid representing pixels. Where as erosion and dilation are considered the primary morphological operations and the operations of opening and closing are secondary operations and these are implemented using erosion and dilation operations. When we subtract the opened image from the original image is known as Top-hat Transform (T_h), which is defined as,

$$T_h = I - (I \circ B).......(3)$$

When we subtract the closed image from the original image (I) is known as Bottom-hat Transform (B_h) and which is defined as,

$$B_h = I - (I \bullet B)........(4)$$

Where, I is the image, B is the Structuring element.

In this proposed work first, we read the original grayscale image. Then add this image to the top hat transform and then subtract the bottom-hat transform i.e. we perform bottom hat & tophat operation on input image by using disk, diamond, square and octagon shaped structuring element. Then adjust intensity of the image and repeat both procedures. After that, we extract the background of the image by using morphological opening operation having disk, diamond, square & octagon shaped structuring element. Here we have used structuring element, which has to changes the radius (up to 3, 6 - 9) that found good to get satisfied result and then subtract the background from the adjusted image and get final result, which are satisfied and promising. But these all combinations of disk-disk, disk-diamond, diamond-diamond, diamond-square, octagon-disk, octagon-octagon of structuring elements are different types of statistical results are found. In this paper we have deals with morphological segmentation technique for various images. Segmentation by using mathematical morphology, which is very attractive because it efficiently deals with geometrical features such as size, shape [5,13], contrast, or connectivity that can be considered as segmentation oriented features. The basic features of structuring elements are, square specify width of S.E., such as 'w' must be non-negative integer scalar, Diamond specify the distance from the S.E., radius 'R' must be non-negative integer scalar, Disk specifies periodic-line S.E., radius 'R' must be non-negative integer & Octagon as measured along the horizontal and vertical axis, radius 'R' must be non-negative multiple of 3.

Mean

$$m = \sum_{i=0}^{L=1} z_i p(z_i) \tag{5}$$

Standard Deviation

$$\sigma = \sqrt{\mu_2(z)} = \sqrt{\sigma^2} \tag{6}$$

Mean is to measure a average intensity and Standard deviation is to measure average contrast. Where, z_i is a random variable indicating intensity, p (z) is the histogram of the intensity levels in a region; L is the number of possible intensity levels.

Proposed Algorithm

1. Read the original grayscale image.
2. To use the S.E. disk, diamond, square & octagon for preprocessing.
3. Subtract the background.
4. Add the transform for postprocessing.
a) Top hat for contrast enhances.
b) Bottom hat for find intensity.
5. Calculate Mean & STD & object count statistical parameter.

Result and Discussion

In this paper, we have applied new morphological technique to determine the spot by segmenting the background and noisy region of the image, which gets spot identification easy as well as we can easily observe the increase in number of spot. For this experiment we have taken microarray images and worked on it, out of that the Fig. 1 is the original image, which is the problem in microarray and Fig. 2 and 3 is the resultant microarray images. We have compared the spots before and after applied the proposed technique respectively. As mention the mathematical morphology techniques are useful for microarray segmentation but in this spot identification process structuring element of shapes and radius is a change at various times and we seen various types of results visually and statistically. Also we analyze number of 29 different S.E. combinations with 80 different microarray images. Generally microarray images are contains number of spots is circular shape but we use four types of structuring elements shapes such as disk, diamond, square, octagon & also to change the radius up to 3 to 9 as shown table 1,2 & graph1,2. First we check the shape is octagon-octagon and radius is 3-3, 3-6,…,3-9, etc. & then change shapes octagon-diamond, octagon-disk, square-octagon as like this & then observe which techniques combination gives best result. The overall performance is best for square-octagon having radius (3,9) because by using morphology it separate foreground and background but could not separates two spots clearly. In this experiment results are poor than our previous disk, diamond & square shapes structuring element method, but in object count the number of objects is increased because of morphology the objects is separated from each other. Another fact has been detected is the radius of the structuring element always must be power of three. In comparison of other techniques, such as watershed segmentation many others have mentioned this is very useful to microarray images. But we have checked the results using such technology this is could not workout for microarray images result as shown in Fig. 3.

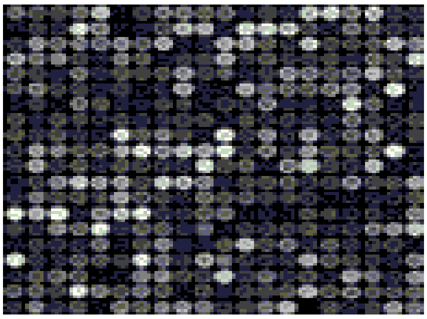


Fig. 1- Original image

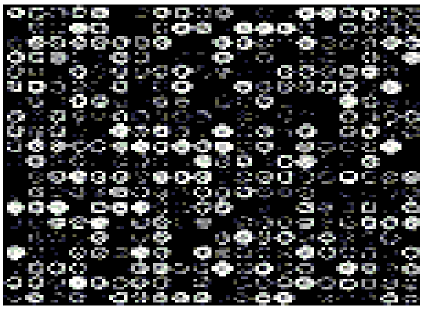


Fig. 2- square-octagon [3,9]

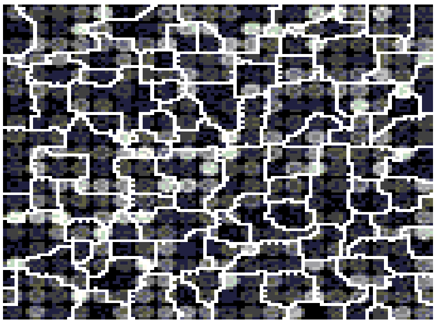


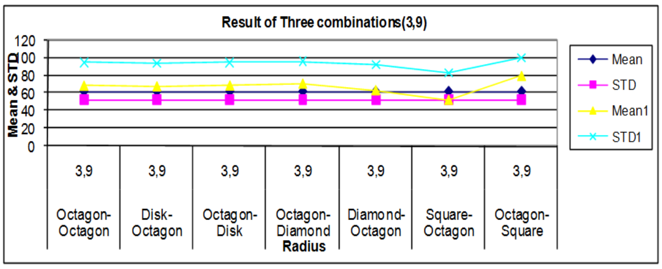
Fig. 3- Using-watersh

Table 1- Result of microarray image (Original & Resultant) using four shapes disk-diamond-square-octagon

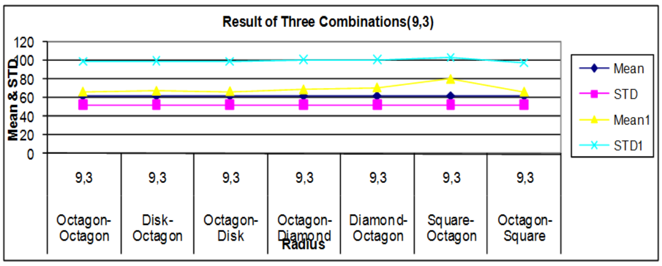
Combination of S.E.	Radius	Original Image			Resultant image		
		Mean	STD	No (object)	Mean	STD	No (object)
Octagon-Octagon	3,9	62.0736	52.7239	369	68.691	95.4744	579
Disk-Octagon	3,9	62.0736	52.7239	369	67.9902	94.4757	610
Octagon-Disk	3,9	62.0736	52.7239	369	68.9765	95.6547	585
Octagon-Diamond	3,9	62.0736	52.7239	369	71.147	96.8122	600
Diamond-Octagon	3,9	62.0736	52.7239	369	63.6527	93.3053	592
Square-Octagon	3,9	62.0736	52.7239	369	52.5249	83.6604	724
Octagon-Square	3,9	62.0736	52.7239	369	79.9404	100.7331	620

Table 2- Result of microarray image (original & Resultant) using four shapes disk-diamond- Square-octagon

Combination of S.E.	Radius	Original Image			Resultant image		
		Mean	STD	No (object)	Mean	STD	No (object)
Octagon-Octagon	9,3	62.0736	52.7239	369	66.9085	99.322	557
Disk-Octagon	9,3	62.0736	52.7239	369	67.7772	99.6987	565
Octagon-Disk	9,3	62.0736	52.7239	369	67.4024	99.2903	555
Octagon-Diamond	9,3	62.0736	52.7239	369	69.3603	101.004	532
Diamond-Octagon	9,3	62.0736	52.7239	369	71.5275	101.047	567
Square-Octagon	9,3	62.0736	52.7239	369	80.9266	103.1635	610
Octagon-Square	9,3	62.0736	52.7239	369	66.801	97.9506	615



Graph 1- Graph shows x-axis contains Shapes & Radius and y-axis contains Mean & STD



Graph 2- Graph shows x-axis contains Shapes & Radius and y-axis contains Mean & STD

Conclusion and Future Work

In this paper, we have given the results of morphological segmentation technique on microarray image for detecting the spot; it improves the number of gene position determination. As shown in the results these techniques work well for microarray spot detection than the other existing techniques. In this experiment we use these four shapes but overall performance is poor by using octagon-octagon shapes & having radius is 3-9, but this good as compare to old technique such as fixed circle, watershed segmentation, etc. Now, also we use new object count statistical parameter this is very fast and automated. In future work, we compare previous and present combinations & checkout which is best for overall and also try to improve the present drawbacks & check the other parameters such as low contrast image, bright contrast & high contrast image & also observes the changes between these all combinations of previous & present and finally concludes which is a best method is used to produce an automated, fast and simple gene identification system. And use this technique to other applications such as MRI images, etc.

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