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Morphological Spot Detection and Analysis for Microarray Images

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Abstract*— DNA microarray technology has promised a very accelerating research inclination in recent years. There are numerous applications of this technology, including clinical diagnosis and treatment, drug design and discovery, tumor detection, and in the environmental health research. Enhancement is the major pre-processing step in microarray image analysis. Microarray images when corrupted with noise may drastically affect the subsequent stages of image analysis and finally affects gene expression profile. Spot detection is the major preprocessing stage in microarray image segmentation. In this paper, morphological approach to detect spots in a subgrid. The proposed approach consists of two phases. First phase is morphological preprocessing, second phase includes spot detection model uses bottomhat transform. Experiments on Stanford, TBDB and UNC database illustrate robustness of the proposed approach in the presence of noise, artifacts and weakly expressed spots. Experimental results and analysis illustrates the performance of the proposed method with the contemporary methods discussed in the literature.

***Keywords*—morphology, dilation, erosion, bottomhat transform.**

I. INTRODUCTION

Microarray technology has emerged as a widely used tool in genomic research, it allows for simultaneously measuring the levels of thousands of different RNA molecules expressed from various genes. Studying these measurements facilitates understanding the biological processes present in living and nonliving organisms. One application of microarray technology deals with identifying genes with different expression levels under different conditions or at different time points. DNA microarray is a powerful tool that allows biologists to monitor expression levels of thousands of genes simultaneously by using an array of DNA molecules that allow many hybridization experiments to be performed in parallel. The cDNA microarray is a popular and effective method for simultaneous assaying the expression of large numbers of genes and is perfectly suited for the comparison of gene expression in different populations of cells. The basis of DNA microarrays is the construction of high density arrays of spots on glass slides that are hybridized with fluorescently labeled mRNA-derived targets. A microarray refers to the physical substrate to which bio sequence reports (cDNA or oligos) are attached.

Microarrays are hybridized with labeled samples and then scanned and analyzed to generated data. Hybridization is the act of treating a microarray with one or more labeled preparations from specified set of conditions. Microarrays exploit the preferential binding of complementary single-stranded nucleic acid sequences. The goal of most microarray experiments is to survey patterns of gene expression by assaying the expression levels of thousands or more genes in a single assay, instead of working on a gene-by-gene basis. There may be tens of thousands of spots on an array, each spot containing a huge number of identical DNA molecules (or fragments of identical molecules), of lengths from twenty to hundreds of nucleotides. For gene expression studies, each of these molecules ideally should identify one gene or one exon in the genome; however, in practice this is not always so simple and may not even be generally possible due to families of similar genes in a genome. Also, one can be confused by the complexity of data analysis.

Microarray image processing consists of the following sequence of three stages 1. Gridding, separation of spots by assignment of image coordinates to the spots. 2. Segmentation, separation between the foreground and background pixels and 3.Intensity extraction, computation of the average foreground and background intensities for each spot of the array.

Microarrays have found numerous applications in the medicine and biology fields. Some of them are:Gene discovery: DNA Microarray technology helps in the identification of new genes, know about their functioning and expression levels under different conditions. Disease diagnosis: DNA Microarray technology helps researchers learn more about different diseases such as heart diseases, mental illness, infectious disease and especially the study of cancer. Drug discovery: Microarray technology has extensive application in Pharmacogenomics. Pharmacogenomics is the study of correlations between therapeutic responses to drugs and the genetic profiles of the patients. Toxicological research: Microarray technology provides a robust platform for the research of the impact of toxins on the cells and their passing on to the progeny.

II. RELATED WORK

The literature survey carried out has revealed that a fair amount of research has been put in the areas of microarray image segmentation. Virgnie Mittard-Runte [1] has explained the process of microarray preparation, image acquisition and analysis, data pre-processing and normalization and data analysis of microarray images.

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Rastislav Lukac [2] have proposed a vector processing based framework suitable for cDNA microarray image segmentation is introduced and analyzed in this paper.
By using nonlinear, generalized selection vector filters the framework proposed here classifies the cDNA image data as either microarray spots or image background. Emmanouil Athamasiades [3] have proposed a new methodology for the segmentation of microarray images which is based on the combination of Gaussian Mixture Models (GMM) with gradient vector flow (GVF). Eleni Zacharia [4] has proposed an original and fully automatic approach for accurately locating a distorted grid structure in a microarray image is presented. The gridding process is expressed as an optimization problem which is solved by using a genetic algorithm (GA). The GA determines the line-segments constituting the grid structure. The proposed method has been compared with existing software tools as well as with a recently published technique. Emmanouil Athanasiadis [5] have proposed a method which is combination of Stationary Wavelet Transform (SWT) and Marko Random Field (MRF), which is called Wavelet Marko Random Field (WMRF). Emmanouil I. Athanasiadis, [6] have proposed segmentation ability of the fuzzy Gaussian mixture model (FGMM) clustering algorithm, applied on complementary DNA (cDNA) images. Shenghua NI [7] has proposed segmentation method based on ACWE with applications in cDNA segmenting. Emmanouil I. [8] has proposed a Markov random field (WMRF) model for segmenting complementary DNA (cDNA) microarray images. Eleni Zacharia [9] has proposed an original and fully automatic approach to accurately segmenting the spots in a cDNA microarray image is presented. In order for the segmentation to be accomplished, each real spot of the cDNA microarray image is represented in a three-dimensional (3-D) space by a 3-D spot model. A.Sri Nagesh [10] has proposed a methodology to investigate the accuracy of spot segmentation of a microarray image, using morphological image analysis techniques, watershed algorithm and iterative watershed algorithm. Dimitris Bariamis [11] have proposed a M3G, a novel method for automatic gridding of cDNA microarray images based on the maximization of the margin between the rows and the columns of the spots. S.Raghavarao [12] has proposed a new image segmentation algorithm based on the hard version of the information bottleneck method is presented. Yan Yang [13] has proposed a model-based segmentation method, our procedure is able to identify inner holes, fuzzy edges and blank spots that are common in microarray images. The approach is independent of microarray platform and applicable to both single- and dual channel microarrays. Richard A Moffitt [14] has proposed a caCORRECT is shown to improve the accuracy of gene expression, and the reproducibility of experimental results in clinical application. This study suggests that caCORRECT will be useful to clean up possible artifacts in new as well as archived microarray data. Luis Rueda [15] have proposed a parameterless and fully automatic approach that first detects the sub-grids given the entire microarray image, and then detects the locations of the spots in each sub-grid. Nagaraja .J [16] have proposed an approach that achieves an automated way for applying mathematical morphology for the enhancement of microarray images. Satish Viswanath [17] have proposed a novel framework termed consensus embedding which leverages ensemble classification theory within dimensionality reduction, allowing for application to a wide range of high-dimensional biomedical data classification and segmentation problems. Andrew Janowczyk [18] has proposed a system for accurately quantifying the presence and extent of stain on account of a vascular biomarker on tissue microarrays. Saiful Islam [19] has proposed four Edge detection techniques for natural image segmentation and they are Roberts Edge detection, Sobel Edge detection, Prewitt Edge detection, and LoG Edge detection.

III. PROPOSED APPROACH

A. *Microarray Segmentation model*

In this section we are going to discuss about microarray image segmentation model using mathematical morphology. Segmentation is the major step in microarray image analysis which includes spot detection and analysis. Figure 1 shows dataflow for the spot detection for the microarray images.

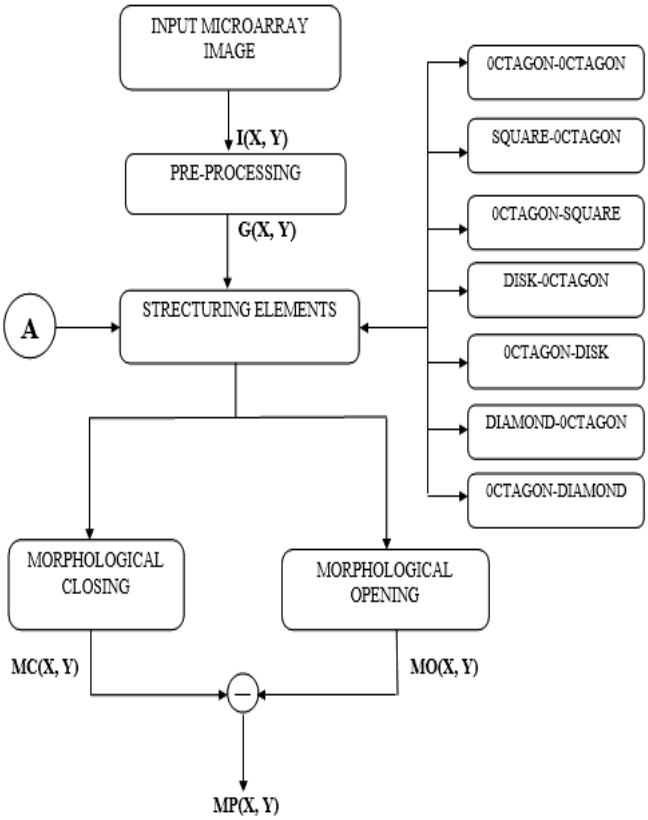

Fig 1: Dataflow diagram of Proposed Approch

Input RGB microarray image I(x,y) to be preprocessed to get gray scale image G(x,y).So preprocessed image G(x,y) will be morphology operated on different Structuring Element.

Structuring element is 1still the key factor of morphology operations. Applying structuring elements with different radius leads to diverse results of analyzing and processing of geometric characteristic.

Morphological preprocessing includes morphological opening MO(x, y) and morphological closing MC(x, y).

$$MP(x, y) = MC(x, y) - MO(x, y) \quad (1)$$

Where x varies from one to rows and y varies from one to column.

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Morphological opening is erosion followed by dilation.

$$G(x, y) \bullet SE = (G(x, y) \oplus SE) \Theta SE \quad (2)$$

Morphological closing is dilation followed by erosion.

$$G(x, y) \circ SE = (G(x, y) \Theta SE) \oplus SE \quad (3)$$

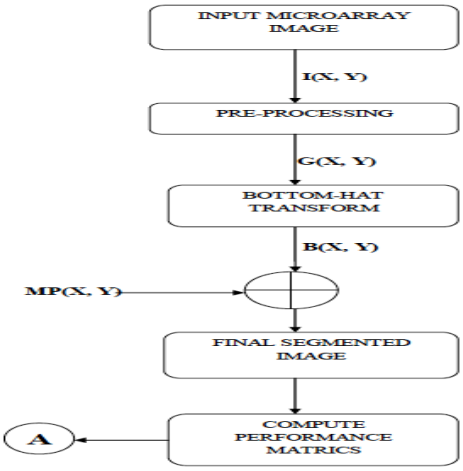

Fig 2: Dataflow diagram of Bottom-hat transform

Dilation is an operation that grows or thickens: objects in a gray scale image. The specific manner and extent of thickening is controlled by a shape referred through structuring element. Erosion shrinks or thins objects in a gray scale image. The manner and extent of shrinking is controlled by a structuring element.

The final segmented image is obtained by adding Bottom hat Transform image BT(x, y) with Morphological preprocessed image MP(x,y). The Bottom hat transform is performed on G(x,y).It is dilation operation followed by erosion operation.

$$B(x, y) = G(x, y) - (G(x, y) \bullet SE) \quad (4)$$

$$G(x, y) \bullet SE = ((G(x, y) \oplus SE) \Theta SE \quad (5)$$

In this proposed work first, we read the original grayscale image then do start further analysis. In next step subtract the bottom-hat transform i.e. we perform bottom hat operation on input image by using square, 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 square & octagon shaped structuring element. Here we have used structuring element, whose radius changes the radius (up to 1 - 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 square-octagon, octagon-square, disk-octagon, diamond-octagon of structuring elements are different types of statistical results are found. As this paper deals with morphological segmentation technique for various image segmentation mathematical morphology, which is very attractive because it efficiently deals with geometrical features such as size, shape [3,9], contrast, or connectivity that can be considered as segmentation oriented features. The basic features of structuring elements are, Square specifies periodic-line S.E., radius 'R' must be non-negative integer & Octagon specify the distance from the S.E., radius 'R' must be nonnegative integer scalar.

Mean:

$$\bar{I}_i = \frac{1}{N} \sum_{j=1}^{N} I_{ij} \quad (6)$$

Standard Deviation:

$$\sigma(i) = \frac{1}{M-1} \sum_{j=1}^{N} ((I_{ij} - \bar{I}_i)^2)^{\frac{1}{2}} \quad (7)$$

Mean is to measure an average intensity and Standard deviation is to measure average contrast. Where, *N* and *M* are the image numbers of rows and columns, respectively, *i* and *j* are the indices of the image columns and rows, respectively. I*ij* is the intensity of the pixel located at column *i* and row *j*.

IV. 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.4 is the original image, which is the problem in microarray, and fig. 5 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 as shown in table 1 & fig. 5 shows Mean & STD Vs Shape & radius. Also we analyze number of 8 combinations of S. E. & 80 different microarray images. Generally microarray images is contains number of spots is circular but we use two types of structuring elements shapes such as octagon, square & also to change the radius up to 1to9 as shown in table 1. Firstly we check the shape is octagon-octagon and radius is 3-3, 3-6…3-9, etc. & then change shapes square-octagon, octagon-square, disk-octagon, diamond-octagon, as like this. It is also found that visibility of the image and clarity is better. And statistically the calculated mean and STD before and after is also good than other combination, because by using morphology it separate foreground & background but spots are could not clearly separated. In addition we have check one additional parameter that is new one (object count), by using this the number of spots is increased as compare to manual count; because of morphology objects are clearly separated from each other, in this case it is considered to the false rejection rate. Fig. 3 shows the original microarray image and Fig. 4 shows the resultant microarray image.

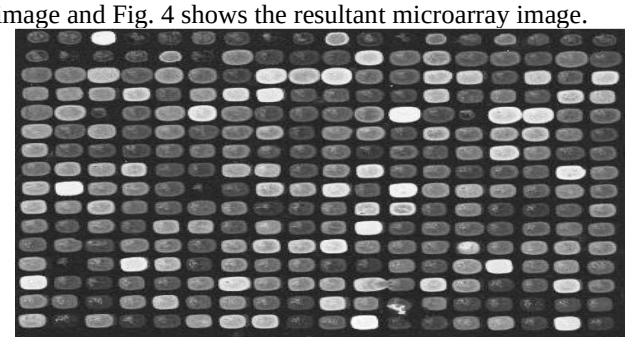

Fig 3: Original Microarray image, Image ID: 75530, Database: TBDB

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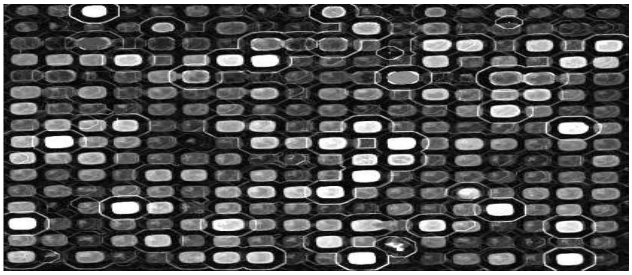

Fig 4: Resultant Microarray image Square-Octagon (3, 9), Image ID: 75530, Database: TBDB

Table: 1: Result of microarray image (original & Resultant) using two shapes Square, Octagon, Disk and Diamond.

Combination of S.E	Radius	Segmented Image		
		Mean	STD	No. of Connected Components
Octagon-Octagon	3,9	54.2175	6.869	249
Square-Octagon	3,9	40.4807	9.1057	259
Octagon-Square	3,9	39.9231	7.3347	204
Disk-Octagon	3,9	48.8071	7.7359	254
Octagon-Disk	3,9	46.187	5.295	215
Diamond-Octagon	3,9	55.2986	7.249	345
Octagon-Diamond	3,9	51.2484	6.0459	269

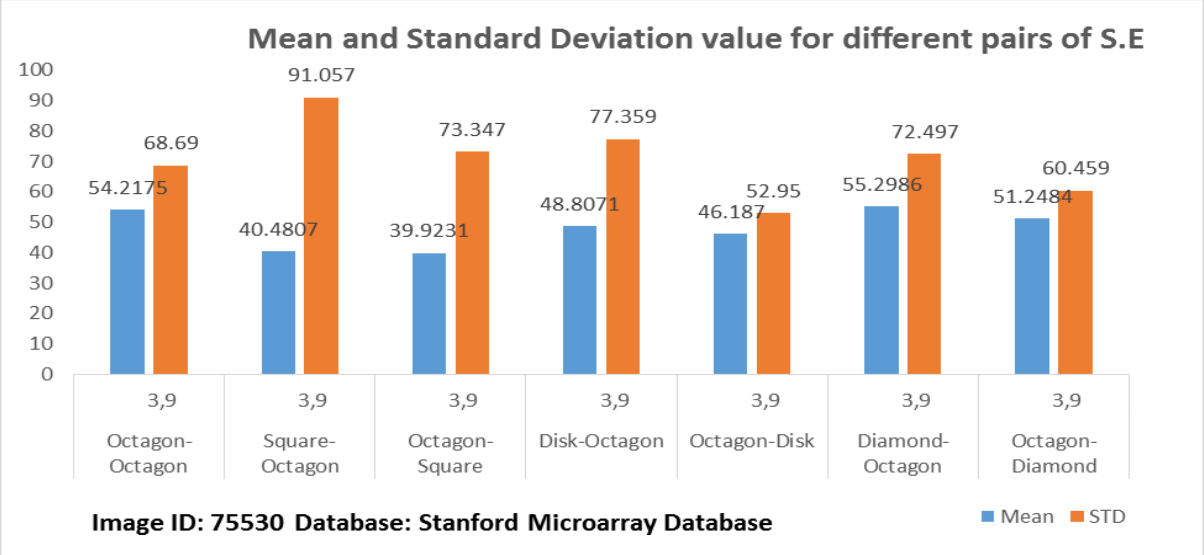

Fig 5: Graph shows x-axis contains Shapes and y-axis contains Mean & STD.

V. CONCLUSION

In this work an approach for spot detection for microarray image is presented. The spot detection is performed through closing, opening and bottom-hat transform which are implemented using morphological dilation and erosion. From the experimental results it has been observed that overall performance is best for square-octagon having radius (3,9). The entire process is robust, in the presence of noise, artifacts and weakly expressed spots. The proposed work can be used at pre-processing phase in microarray image analysis before using it in any of the stages of microarray image analysis, which then results in accurate gene expression profiling.

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