A Wavelet Based Morphological Approach for Enhancement of Microarray Images

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Abstract— DNA microarray technology is a popular area of research in recent years. This technology plays a major role in the field of clinical diagnosis, plant biotechnology, drug discovery, gene discovery and many applications. Enhancement is a major pre-processing step in microarray image analysis. This paper presents an approach for enhancement of microarray images using biorthogonal wavelet and mathematical morphology. Experiments on Stanford, TBDB and UNC database illustrate robustness of proposed approach in presence of noise, artifacts and weekly expressed spots. Experimental results and analysis illustrates the performance of proposed method with the contemporary methods discussed in the literature.

Index Terms— Microarray, Top-hat transform, Bottom-hat transform, Wavelet decomposition, Mathematical morphology.

I. INTRODUCTION
The DNA microarray is a popular and effective method for simultaneous as saying the large expression of large number of genes and perfectly suited for the comparison of gene expression in different population of cells. DNA microarray technology has a large impact in many application areas, such as diagnosis of human diseases and treatments (determination of risk factors, monitoring disease stage and treatment progress, etc.), agricultural development (plant biotechnology), and quantification of genetically modified organisms, drug discovery, and design.

A microarray is a collection of spots containing DNA deposited on the surface of a glass slide. Each of the spots contains multiple copies of single DNA sequence. The probes are tagged with DNA florescent molecules which emit detectable light when stimulated by laser. The emitted light is captured by detector which records light intensity. When laser scans the slide a large image containing thousands of spots produced. Microarray image analysis consists of three basic steps which are gridding, segmentation and information extraction. Gridding is the process of identifying the area in the image and assigning the image to each spot. Segmentation, separation between the foreground and background pixels and information extraction, computation of the average foreground and background intensities for each spot of the array. Information is extracted by foreground and background pixel intensities. DNA microarray technology is frequently used to monitor the changes in gene expression for thousands of genes simultaneously. This technology has empowered the biological society to explore the genetic causes of anomalies occurring in the functioning of the human body.

Microarray image may contain different sources of errors. Such as electronic noise, dust on slide, photon noise and other sources causes high level of noise, in which may propagate through higher image analysis
leading to difficulty in identifying the genes that each type of cells is expressing to draw accurate biological conclusions.

Spot recognition is a complicated task as microarray image gets corrupted by noise sources during image acquisition also bright artifacts may be detected incorrectly as spots of microarray image. Hence it is very much essential to remove the noise present in the image. The image enhancement is necessary to improve the interpretability of information in images to provide better input for the higher image processing applications. Low quality images are thus to be enhanced by appropriate methods to interpret the accurate expression levels. Image enhancement improves the image quality by refining the image with respect to structural content, statistical content, edges, textures and presence of noise. It can be further used for accurate measurement of gene expression profiling.

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 enhancement. X. H. Wang, Robert S. H. Istepanian and Yong Hua Song [4] have proposed a new approach based on wavelet theory to provide a Denoising approach for eliminating noise source and ensure better gene expression. Method of denoising applies stationary wavelet transform to pre-process the microarray images for removing the random noises. Rastislav Lukac and Bogdan Smolka [5] have proposed novel method of noise reduction, which is capable of attenuating both impulse and Gaussian noise while preserving and even denoising the sharpness of the image edges. R. Lukac, et.al [6] have proposed vector fuzzy filtering framework to denoise cDNA microarray images. This method adaptively determines weights in the filtering structure and provides different filter structures. Noise removal using smoothing of coefficients of highest sub bands in wavelet domain is described by Mario Mastrianian and Alberto E. Giraldez [7].

Denoising switching scheme based on the impulse detection mechanism using peer group concept is discussed by N. Plataniotis et.al [8]. A two-stage approach for noise removal that processes the additive and the multiplicative noise component, which decomposes the signal by a multi resolution transform, is described by HaraStefanou, Thanasis Margaritis, Dimitris Kafetzopoulos, Konstantinos Marias and Panagiotis Tsakalides [9]. Guifang Shao, Hong Mi, Qifeng Zhou and Linkai Luo [10] have proposed a new algorithm for noise reduction which included two parts: edge noise reduction and highly fluorescence noise reduction. Ali Zifan, Mohammad Hassan Moradi and Shahriar Gharibzadeh [11] have proposed an approach using of decimated and undecimated multi wavelet transforms. Denoising of microarray images using the standard maximum a posteriori and linear minimum mean squared error estimation criteria is discussed by Tamanna Howlader et.al [12]. J.K.Meher et.al [13] have proposed novel pre-processing techniques such as optimized spatial resolution (OSR) and spatial domain filtering (SDF) for reduction of noise from microarray data and reduction of error during quantification process for estimating the microarray spots accurately to determine expression level of genes. Weng Guirong has proposed a novel filtering method to denoise microarray images using edge enhancing diffusion method [14].

Factorial analysis on simulated microarray images to study the effects and interaction of noise types at different noise levels is discussed by Yogananda Balagurunathan et.al [15]. Chitra Gopalapppa et.al [16] have proposed a novel methodology for identification and scanning noise from microarray images using a dual tree complex wavelet transform. A two phase scheme for removing impulse noise from microarray images by preserving the feature of interest is discussed by Rammurugesan et.al [17]. Arunakumari Kakumani et.al [18] have proposed a method to denoise microarray images using independent component analysis. Enhancement approach which uses principles of fuzzy logic in conjunction with data adaptive filter to enhance noisy microarray images is presented by Rastislav Lukac et.al [19]. Wang li-qianget.al [20] presents a novel method to reduce impulse noise by employing the switching scheme which uses differences between the standard deviation of the pixels within the filter window and the current pixel of concern. Nader Suffarian et.al [21] have proposed an approach which is implemented as conditional sub-block bi-histogram equalization (CSBE) which has the ability to improve the gridding results in DNA microarray analysis.

Most of the methods proposed by researchers have various assumptions on factors such as type of thresholding used, parametric assumptions and decomposition levels, which in turn leads to misclassification of foreground pixels from the background pixels in the segmentation process and
finally affects gene expression profile. This is very essential at the pre-processing stage because in 
the microarray image analysis each stage affects subsequent stage, so that an accurate biological 
conclusion can be drawn. Denoising of microarray image is a challenging task in the pre-
processing step of microarray image analysis. So, techniques without the above mentioned 
constraints and which depends exclusively on the image characteristics is in demand. Figure 1. 
shows a subgrid of microarray image.

Fig 1.Subgrid of a microarray image

III. PROPOSED ENHANCEMENT MODEL

This section presents a description of proposed enhancement model. It consists of two methods, one is 
mathematical morphology and wavelet concepts. The input for model is noisy microarray image I(x,y). 
Where x and y are the size of the image. Pre-processing step is consists of two tasks, one is to convert image 
from RGB to binary image and selecting structuring element for morphological operations. In this approach 
bull non flat structuring element is selected with radius 30 and height 17.

Morphological operations such as top hat and bottom hat transformation are performed on The processed 
image G(x,y).Top-hat transform(TH) is performed on G(x,y) and it is the difference between G(x,y) and its 
opening by structuring element (SE).Morphological opening is erosion followed by dilation. The resultant 
image will be TH(x,y).

\[
(TH(x, y) = (G(x, y) - (G(x, y) \circ SE)) \quad (1)
\]
\[
(G(x, y) \circ SE = ((G(x, y) \Theta SE) \oplus SE)) \quad (2)
\]

Bottom hat transform (BH) is performed on G(x,y). BH is the difference between closing by structuring 
element (SE) and G(x,y).Morphological closing is dilation followed by erosion. The resultant image will be 
BH(x,y).

\[
(BH(x, y) = ((G(x, y) \bullet SE) - G(x, y))) \quad (3)
\]
\[
(G(x, y) \bullet SE = ((G(x, y) \oplus SE) \Theta SE)) \quad (4)
\]
Where $\oplus$ and $\ominus$ denote dilation and erosion respectively. $\circ$ and $\bullet$ denote opening and closing respectively. The image $E(x,y)$ is obtained by the following manipulation of morphological images.

$$E(x,y) = TH(x,y) + G(x,y) - EH(x,y)$$  \hspace{1cm} (5)

Computation of pixel threshold (PT) includes the following steps.
1. Calculate number of 8-adjacent connected components
2. Find total number of pixels in all connected components (SC)
3. ($PT = SC / y$)

The decomposition vector and the corresponding bookkeeping matrix is obtained by applying 2-D wavelet decomposition at level 2 using biorthogonal wavelet to obtain $PT(x,y)$. Soft threshold (WT) is estimated using coefficients generated by decomposition process. Multilevel 2-D wavelet reconstruction is performed on $WT(x,y)$ to obtain enhanced image ($WR(x,y)$). Figure 2 shows dataflow diagram of proposed approach.

The following chart describes the decomposition steps:
Two-dimensional DWT leads to a decomposition of approximation coefficients at level $j$ in four components: the approximation at level $j+1$, and the details in three orientations (horizontal, vertical, and diagonal).
CA0=S [bookkeeping matrix] for the decomposition initialization, so for $j=2$ the two-dimensional wavelet tree has the form shown in Figure 3.

Fig 3. The two-dimensional wavelet trees

where $A, H, V, D,$ are row vectors such that $A =$ approximation coefficients $H =$ horizontal detail coefficients $V =$ vertical detail coefficients $D =$ diagonal detail coefficients Each vector is the vector column-wise storage of a matrix.
IV. EXPERIMENTAL RESULTS AND ANALYSIS

In this section, the performance of the proposed approach is evaluated on real noisy microarray images drawn from SMD (Stanford microarray database), UNC (University of North California microarray database) and TBDB database. The images are available for free download from https://genome.unc.edu, http://www.tbdb.org/cgiin/data/clickable.pl.html  

Figure 4 shows noisy microarray image and in Figure 5 Enhanced image using proposed approach is shown.

Fig 4. Noisy subgrid, Image ID: 39119, Database: TBDB

Fig 5. Enhanced subgrid, Image ID: 39119

To quantify both the degree of filtering as well as the improvements due to enhancement algorithms, various performance measures are used. Such as mean squared error (MSE), peak signal to noise ratio (PSNR) in dB and contrast improvement index (CII).

Mean square error can be calculated using equation 6.

\[ \text{MSE} = \sum_{i=0}^{N-1} \sum_{j=0}^{M-1} [X(i,j) - Y(i,j)]^2 \]  

\[ (6) \]

Where X and Y are two arrays of size NxM

The PSNR value can be computed using equation 7.
\[ \text{PSNR} = 10 \log_{10} \frac{L^2}{MSE} \]  

(7)

$L$ reflects the range of values that a pixel can take. Higher the peak signal to noise ratio value higher is the quality of the image and lower the mean squared value higher is the image quality. Here we have compared the performance of different filters. Contrast improvement index, which is the ratio of mean background and foreground of the enhanced image. Better clarity will be achieved if CII is near to one.

**TABLE I:** MSE, PSNR and CII for different images obtained using proposed approach

<table>
<thead>
<tr>
<th>Image Id</th>
<th>Mean Square Error(MSE)</th>
<th>Peak Signal Noise Ratio(PSNR)</th>
<th>Contrast Improvement Index</th>
</tr>
</thead>
<tbody>
<tr>
<td>17931</td>
<td>4.40</td>
<td>84.04</td>
<td>0.8345</td>
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<tr>
<td>31919</td>
<td>3.878</td>
<td>72.08</td>
<td>0.7976</td>
</tr>
<tr>
<td>35964</td>
<td>3.743</td>
<td>74.86</td>
<td>1.0000</td>
</tr>
<tr>
<td>42247</td>
<td>4.2614</td>
<td>85.22</td>
<td>0.9906</td>
</tr>
</tbody>
</table>

**TABLE II:** Comparison of MSE, PSNR and CII with existing filters

<table>
<thead>
<tr>
<th>Image id</th>
<th>Existing Filters</th>
<th>Wiener filter</th>
<th>Median Filter</th>
<th>Low pass filter</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>MSE</td>
<td>PSNR</td>
<td>CII</td>
<td>MSE</td>
</tr>
<tr>
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<td>21.43</td>
<td>17.00</td>
<td>0.5787</td>
<td>24.83</td>
</tr>
<tr>
<td>31919</td>
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<td>15.40</td>
<td>0.7868</td>
<td>18.42</td>
</tr>
<tr>
<td>35964</td>
<td>54.04</td>
<td>14.88</td>
<td>0.7133</td>
<td>154.0</td>
</tr>
<tr>
<td>42247</td>
<td>59.56</td>
<td>14.82</td>
<td>0.6943</td>
<td>139.9</td>
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</table>

**CONCLUSIONS**

In this work an approach is presented for enhancement of microarray images. Removal of noise is performed through wavelet decomposition. Mathematical morphology is used to enhance the image. To the enhanced image three image quality assessment parameter like MSE, PSNR and CII is calculated. From the test result entire approach is robust and can be used for pre-processing step for microarray image analysis.

**REFERENCES**


