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SLIC Superpixel based Fuzzy C-means Algorithm for Segmentation of Microarray Images

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Abstract : Microarray technology allows the simultaneous monitoring of thousands of genes in parallel. Based on the gene expression measurements, microarray technology have proven powerful in gene expression profiling for discovering new types of diseases and for predicting the type of a disease. Enhancement, Gridding, Segmentation and Intensity extraction are important steps in microarray image analysis. This paper presents simple linear iterative clustering (SLIC) based Fuzzy c-means (FCM) algorithm for segmentation of microarray image. Superpixels are clusters of pixels which share similar features, thus they can be used as mid-level units to decrease the computational cost in many vision applications. The proposed algorithm utilizes superpixels as clustering objects instead of pixels. The qualitative and quantitative analysis shows that the proposed method produces better segmentation quality than *k*-means, moving *k*-means and fuzzy c-means clustering methods.

Keywords: Empirical Mode decomposition, Simple Linear Iterative Clustering, Fuzzy c-means, Microarray Images, Image Segmentation.

1. INTRODUCTION

The most powerful tool in molecular genetics for biomedical research is Microarray, which allows parallel analysis of the expression level of thousands of genes. The most important aspect in microarray experiment is image analysis. The output of image analysis is a matrix consisting of a measure of intensity of each spot in the image. This measure denotes gene expression ratio (transcription abundance) between the test and control samples for the corresponding gene. The positive expression indicates the over-expression, while negative expression indicates under-expression between the control and treatment genes. The main components in microarray image analysis are localization, segmentation and spot quantification [1]. The main applications of microarray technology are Gene discovery, Drug discovery, Disease diagnosis, Toxicological research etc [2]. The microarray image analysis is shown in figure 1.

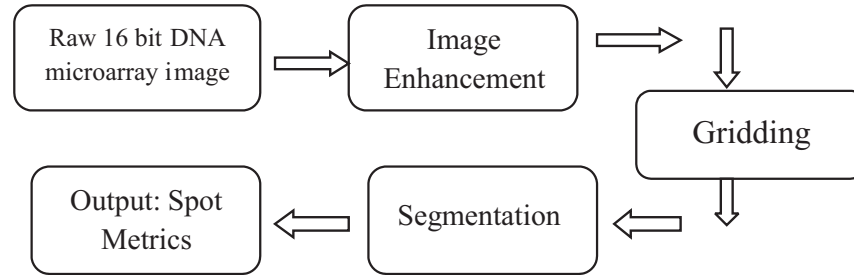


Figure 1: Microarray Image Analysis

The evaluation of microarray images is a difficult task as the fluorescence of the glass slide adds noise floor to the microarray image [3]. The processing of the microarray image requires noise suppression with minimal reduction of spot edge information that derives the segmentation process. Thus the task of microarray image enhancement is of paramount importance [4]. This paper presents a noise removal in microarray images using Empirical Mode Decomposition [EMD]. The BEMD method [5] decomposes the image into several Intrinsic Mode Functions [IMF], in which the first function is the high frequency component, second function next high frequency component and so on, the last function denotes the low frequency component. The mean filter is applied only to the few first high frequency components leaving the low frequency components, as the high frequency components contain noise. The image is reconstructed by combining the filtered high frequency components and low frequency components. After noise removal, gridding, segmentation and Expression ratio calculation are the important tasks in microarray image analysis. Any noise in the microarray image will affect the subsequent analysis [6].

Many microarray image segmentation approaches have been proposed in literature. Fixed circle segmentation [7], Adaptive circle Segmentation Technique [8], Seeded region growing methods [9] and clustering algorithms [10] are the methods that deal with microarray image segmentation problem. This paper mainly focuses on clustering algorithms. These algorithms have the advantages that they are not restricted to a particular spot size and shape, does not require an initial state of pixels and no need of post processing. These algorithms have been developed based on the information about the intensities of the pixels only (one feature). In this paper, SLIC super pixel based fuzzy c-means clustering algorithm is proposed. The qualitative and quantitative results show that proposed method has segmented the image better than k-means, moving k-means and fuzzy c-means clustering algorithms.

The paper is organized as follows: Section II presents Denoising using Bi-dimensional Empirical Mode Decomposition (BEMD)-mean filter method, Section III presents Gridding mechanism of microarray image, Section IV presents SLIC Superpixel algorithm, Section V FCM Clustering Algorithm, Section VI presents SLIC based FCM algorithm, Section VII presents Experimental results and Section VIII report conclusions.

2. BI-DIMENSIONAL EMPIRICAL MODE DECOMPOSITION-DWT THRESHOLDING METHOD

Empirical mode decomposition [11] is a signal processing method that nondestructively fragments any non-linear and non-stationary signal into oscillatory functions by means of a mechanism called shifting process. These oscillatory functions are called Intrinsic Mode Functions (IMF), and each IMF satisfies two properties, (a) the number of zero crossings and extrema points should be equal or differ by one. (b) Symmetric envelopes (zero mean) interpret by local maxima and minima [12]. The signal after decomposition using EMD is non-destructive means that the original signal can be obtained by adding the IMFs and residue. The first IMF is a high frequency component and the subsequent IMFs contain from next high frequency to the low frequency components. The shifting process used to obtain IMFs on a 2-D signal (image) is summarized as follows:

1. Let $I(x, y)$ be a Microarray image used for EMD decomposition. Find all local maxima and local minima points in $I(x, y)$.
2. Upper envelope $Up(x, y)$ is created by interpolating the maxima points and lower envelope $Lw(x, y)$ is created by interpolating minima points. This interpolation is carried out using cubic spline interpolation method.
3. Compute the mean of lower and upper envelopes denoted by $Mean(x, y)$.

$$Mean(x, y) = \frac{(Up(x, y) + Lw(x, y))}{2} \quad (1)$$

4. This mean signal is subtracted from the input signal.

$$Sub(x, y) = I(x, y) - Mean(x, y) \quad (2)$$

5. If $Sub(x, y)$ satisfies the IMF properties, then an IMF is obtained.

$$IMF_i(x, y) = Sub(x, y) \quad (3)$$

6. Subtract the extracted IMF from the input signal. Now the value of $I(x, y)$ is

$$I(x, y) = I(x, y) - IMF_i(x, y) \quad (4)$$

Repeat the above steps (2) to (6) for the generation of next IMFs.

7. This process is repeated until $I(x, y)$ does not have maxima or minima points to create envelopes.

Original Image can be reconstructed by inverse EMD given by

$$I(x, y) = \sum_{i=1}^n IMF_i(x, y) + res(x, y) \quad (5)$$

The mechanism of de-noising using BEMD-DWT is summarized as follows

1. Apply 2-D EMD for noisy microarray to obtain $IMF_i (i = 1, 2, \dots, k)$. The k th IMF is called residue.
2. The first intrinsic mode function (IMF1) contains high frequency components and it is suitable for denoising. This IMF1 is denoised with mean filter. This de-noised IMF1 is represented with DNIMF1.
3. The denoised image is reconstructed by the summation of DNIMF1 and remaining IMFs given by

$$RI = DNIMF1 + \sum_{i=2}^k IMF_i \quad (6)$$

Where RI is the reconstructed band. The flow diagram of BEMD-DWT filtering is shown in figure 2.

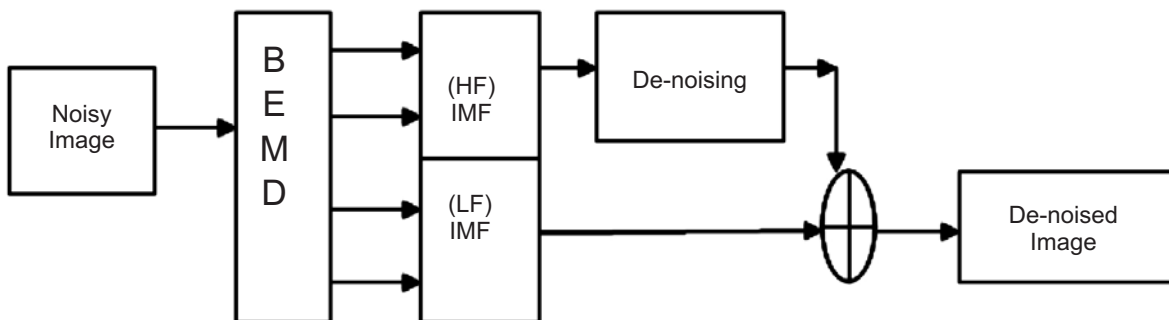


Figure 2: Flow Diagram of BEMD-mean filtering method

3. MICROARRAY IMAGE GRIDGING

Gridding is the process of dividing the microarray image into blocks (sub-gridding) and each block again divided into sub-blocks (spot-detection). The final sub-block contains a single spot and having only two regions spot and background. Existing algorithms for gridding are semi-automatic in nature requiring several parameters such as size of spot, number of rows of spots, number of columns of spot etc. In this paper, a fully automatic gridding algorithm designed in [13] is used for sub-gridding and spot-detection.

4. SLIC SUPERPIXELS

Simple linear iterative clustering (SLIC) is an adaption of k -means for Superpixel generation, with two important distinctions: (1) The number of distance calculations in the optimization is dramatically reduced by limiting the search space to a region proportional to the Superpixel size. This reduces the complexity to be linear in the number of pixels N and independent of the number of superpixels k . (2) A weighted distance measure combines color and spatial proximity, while simultaneously providing control over the size and compactness of the superpixels.

The algorithm of SLIC superpixels generation is given below [14].

1. Initialize k initial cluster centers in $C = [l, a, b, x, y]^T$ by sampling pixels at regular grid steps S .
2. For generation of equal sized super pixels the grid interval S is given by $S = \sqrt{\frac{N}{k}}$
3. Set label $l(i) = -1$ for each pixel i .
4. Set distance $d(i) = \infty$ for each pixel i .
5. For each cluster center C do
6. For each pixel i in a $2S \times 2S$ region around C do
7. Compute the distance D between C and i .
8. The distance D depends on pixel's color (color proximity) and pixel position (spatial proximity), whose values is known. The value of D is given by

$$d_c = \sqrt{(l_j - l_i)^2 + (a_j - a_i)^2 + (b_j - b_i)^2}$$

$$d_s = \sqrt{(x_j - x_i)^2 + (y_j - y_i)^2}$$

$$D = \sqrt{\left(\frac{d_c}{N_c}\right)^2 + \left(\frac{d_s}{N_s}\right)^2}$$

The maximum spatial distance expected within a given cluster should correspond to the sampling interval, $N_s = S$. Determining the maximum color distance N_c is not so straightforward, as color distances can vary significantly from cluster to cluster and image to image. The value of N_c in the range from [1, 40].

9. If $D < d(i)$ then set $d(i) = D$ and $l(i) = k$ got to 6.
10. Goto 5, the same process for each cluster
11. Compute new cluster centers.
12. The clustering and updating processes are repeated until a predefined number of iteration is achieved. The SLIC algorithm can generate compact and nearly uniform superpixels with a low computational overhead.

5. FUZZY C-MEANS CLUSTERING ALGORITHM

The FCM algorithm for segmentation of microarray image is described below [15]:

1. Take randomly K initial clusters from the $m*n$ image pixels.
2. Initialize membership matrix u_{ij} with value in range 0 to 1 and value of $m = 2$.
3. Assign each pixel to the cluster $C_j \{j = 1, 2, \dots, K\}$ if it satisfies the following condition [$D(\cdot, \cdot)$ is the Euclidean distance measure between two values.

$$\begin{aligned} \mu_{ij}^m D(I_i, C_j) &< \mu_{iq}^m D(I_i, C_q), \\ q &= 1, 2, \dots, K \\ j &\neq q \end{aligned} \tag{8}$$

The new membership and cluster centroid values as calculated as

$$\begin{aligned} \mu_{ik} &= \frac{1}{\sum_{j=1}^K \left(\frac{D(C_i, I_k)}{D(C_j, I_k)} \right)^{\frac{1}{m-1}}}, \text{ for } 1 \leq i \leq K \\ C_j^{\wedge} &= \frac{\sum_{j=1}^n u_{ij}^m I_j}{\sum_{j=1}^n u_{ij}^m} \end{aligned} \tag{9}$$

3. Continue 2-3 until each pixel is assigned to the maximum membership cluster [16].

6. SLIC SUPERPIXEL BASED FCM CLUSTERING ALGORITHM

The SLIC algorithm generates superpixels which are used in our clustering algorithm. The superpixels are generated based on the color similarity and proximity in the image plane. The algorithm depends on two values N_s and N_c , the higher the value of N_s corresponds to more regular and grid-like Superpixel structure and lower value of N_c captures more image details. The SLIC Superpixel based FCM clustering algorithm is given below:

1. Generate the superpixels representation of original image, and collect necessary information of superpixels.
2. Initialize cluster centroids $v_i, i = 1, \dots, C$.
3. The objective function F is given by

$$F = \sum_{i=1}^C \sum_{j=1}^Q \gamma_j \mu_{ij}^m \| \xi_j v_i \|^2 + \frac{\alpha}{N_R} \sum_{i=1}^C \sum_{j=1}^Q \mu_{ij}^m \left(\sum_{s_r \in N_j} \gamma_r \| \xi_r v_i \|^2 \right) + \sum_{j=1}^Q \lambda_j \left(1 - \sum_{i=1}^C \mu_{ij} \right) \tag{10}$$

4. The membership values u_{ij} is updated given by

$$\mu_{ij} = \left(\sum_{k=1}^C \left(\frac{\gamma_j \| \xi_j - v_i \|^2 + \frac{\alpha}{N_R} \sum_{s_r \in N_j} \gamma_r \| \xi_r - v_i \|^2}{\gamma_j \| \xi_j - v_k \|^2 + \frac{\alpha}{N_R} \sum_{s_r \in N_j} \gamma_r \| \xi_r - v_k \|^2} \right)^{1/(m-1)} \right)^{-1} \tag{11}$$

Where Q is the number of superpixels in images, γ_j is the number of pixels in superpixel s_j , and ξ_j is the average color value of superpixel s_j . u_{ij} denotes the membership of superpixel s_j to the i th cluster. N_j stands for the set of neighboring superpixels that are adjacent to s_j , and NR is the cardinality of N_j . $\|\cdot\|$ is a norm metric, denoting Euclidean distance between pixels and clustering centroids. The parameter m is a weighting exponent on each fuzzy membership and determines the amount of fuzziness of the resulting classification.

The cluster centroids v_i is updated given by

$$v_i = \left(\sum_{j=1}^Q \mu_{ij}^m \left(\gamma_j \xi_j + \frac{\alpha}{N_R} \sum_{s_r \in N_j} \gamma_r \xi_r \right) \right) \left(\sum_{j=1}^Q \mu_{ij}^m \left(\gamma_j \xi_j + \frac{\alpha}{N_R} \sum_{s_r \in N_j} \gamma_r \xi_r \right) \right)^{-1} \tag{12}$$

6. Repeats Steps 3-4, until $\|V_{new} - V_{old}\| < \epsilon$.

7. EXPERIMENTAL RESULTS

Qualitative Analysis: The proposed clustering algorithm is performed on two microarray images drawn from the standard microarray database corresponds to breast category aCGH tumor tissue. Image 1 consists of a total of 38808 pixels and Image 2 consists of 64880 pixels. Gridding is performed on the input images by the method proposed in [13], to segment the image into compartments, where each compartment is having only one spot region and background. The gridding output is shown in figure 3. After gridding the image into compartments, such that each compartment is having single spot and background, compartment no 1 from image 1 and compartment no 8 from image 2 are extracted. Superpixels are generated for these two compartments using SLIC and segmented using SLIC based FCM algorithm. The Superpixel generation and segmentation is shown in figure 3. **Quantitative Analysis:** Quantitative analysis is a numerically oriented procedure to figure out the performance of algorithms without any human error. The Mean Square Error (MSE) [17] is significant metric to validate the quality of image. It measures the square error between pixels of the original and the resultant images. The MSE is mathematically defined as

$$MSE = \frac{1}{N} \sum_{j=1}^k \sum_{i \in c_j} \|v_i - c_j\|^2 \tag{13}$$

Where N is the total number of pixels in an image and x_i is the pixel which belongs to the j th cluster. The lower difference between the resultant and the original image reflects that all the data in the region are located near to its centre. Table 1 shows the quantitative evaluations of clustering algorithms. The results confirm that SLIC based fuzzy c-means algorithm produces the lowest MSE value for segmenting the microarray image.

Table 1
MSE values

Method	Compartment No 1	Compartment No 8
K-means	96.2	93.8
Moving k-means	92.1	89.2
Fuzzy c-means	83.7	79.9
SLIC FCM	81.8	77.6

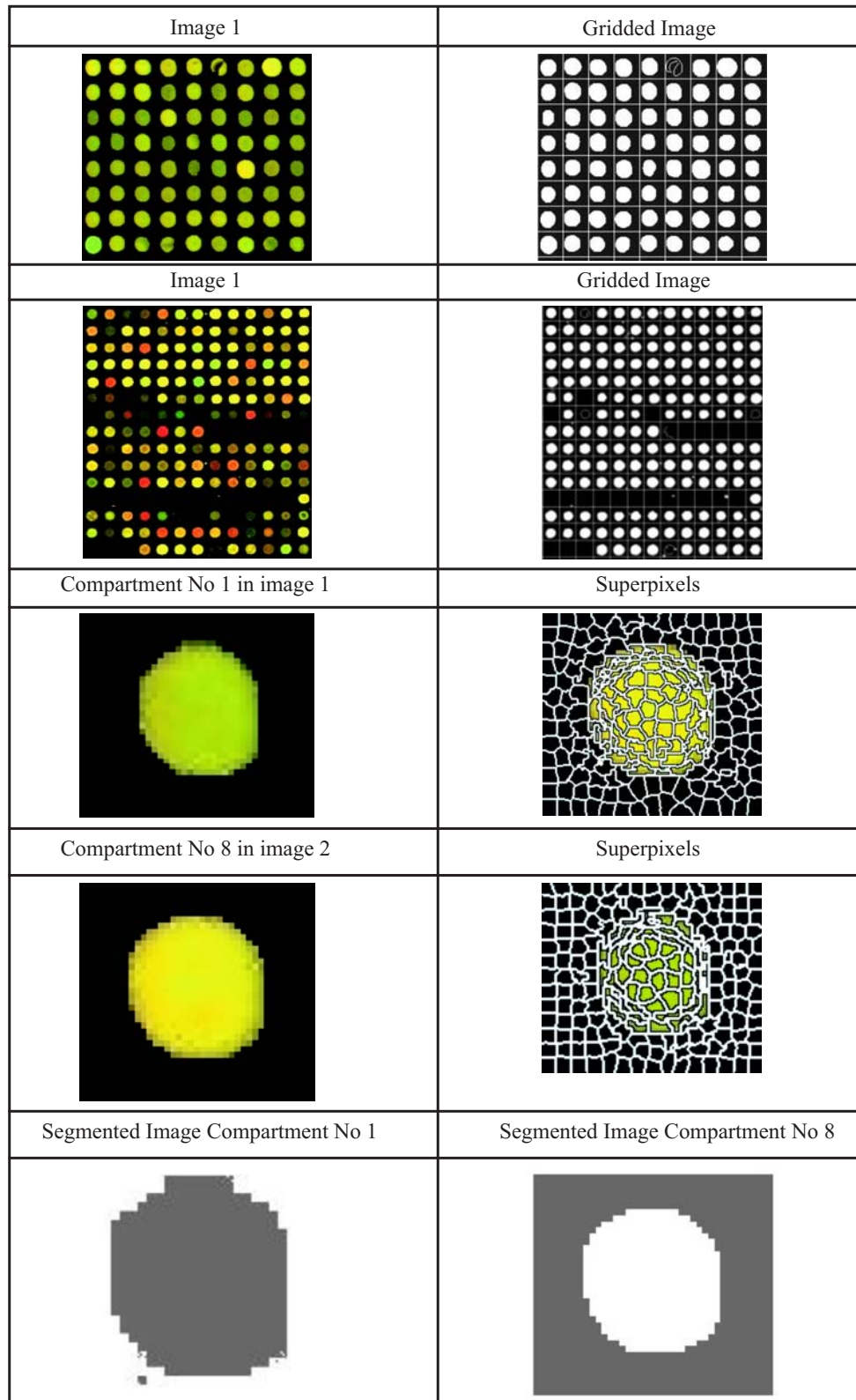


Figure 3: Super pixel based FCM segmentation

8. CONCLUSIONS

Microarray technology is used for parallel analysis of gene expression ratio of different genes in a single experiment. The analysis of microarray image is done with gridding, segmentation and information extraction. The expression ratio of each and every gene spot denotes the transcription abundance between two genes under experiment. This paper presents a new method for microarray image denoising and segmentation. Clustering algorithms have been used for microarray image segmentation with an advantage that they are not restricted to a particular shape and size for the spots. This paper presents SLIC based fuzzy c-means clustering algorithm for segmentation of microarray image. Spot information includes the calculation of Expression Ratio in the region of every gene spot on the microarray image. The expression-ratio measures the transcription abundance between the two sample genes. The proposed method performs better noise suppression and produces better segmentation results.

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