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A Comparative Analysis of Clustering based Segmentation Algorithms in Microarray Images

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Abstract: ***As of now, several improvements have been carried out to increase the performance of previous conventional clustering algorithms for image segmentation. However, most of them tend to have met with unsatisfactory results. In order to overcome some of the drawback like dead centers and trapped centers, in this article presents a new clustering-based segmentation technique that may be able to overcome some of the drawbacks we are passing with conventional clustering algorithms. We named this clustering algorithm as optimized k-means clustering algorithm for image segmentation. OKM algorithm that can homogenously segment an image into regions of interest with the capability of avoiding the dead centre and trapped centre problems. The robustness of the OKM algorithm can be observed from the qualitative and quantitative analyses.***

Keywords: clustering algorithms; dead center problem; Microarray processing; Image segmentation; Microarray processing.

I. INTRODUCTION

Clustering methods have become a revolution step in microarray data analysis because they can identify groups of genes or samples displaying a similar expression profile. Clustering is an unsupervised classification (grouping).attach label to each data points in a set, so that object in each set can share some common trait. I.e. maintaining students (name, Roll-id, Branch, collage name).Microarray technology has been recently introduced and provides solutions to a wide range of problems in medicine, health and environment, drug development, etc. Microarrays, widely recognized as the next revolution in molecular biology, enable scientists to analyze genes, proteins and other biological molecules on a genomic scale [1]. A microarray is a collection of spots containing DNA deposited on the solid surface of glass slide. Each of the spot contains multiple copies of single DNA sequence [2].Microarray expression technology helps in the monitoring of gene expression for tens and thousands of genes in parallel.

A Deoxyribonucleic Acid (DNA) microarray is a collection of microscopic DNA spots attached to a solid surface, such as glass, plastic or silicon chip forming an array. The analysis of DNA microarray images allows the identification of gene expressions to draw biological conclusions for applications ranging from genetic profiling to diagnosis of cancer. The processing DNA microarray image analysis includes three tasks: gridding, segmentation and intensity extraction.

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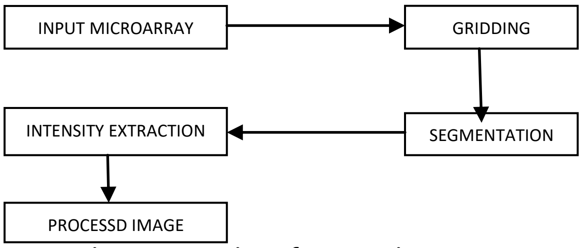

Fig-1: processing of DNA Microarray Image

Gridding: is a crucial process in microarray image processing, in order to locate and identify where exactly the pixel is present. However, this preprocessing ensures addressing spots more efficiently. Gridding method for microarray image is mainly classified into three categories manual, semi-automated and automated. Moreover gridding is the process of segmenting the microarray image into compartments, each compartment having only one spot and background Segmentation: Based on segmentation, the accuracy of microarray data is significantly affected. From fast few years, much number of methods has been adopted for segmentation of microarray images. Fixed and adaptive circle segmentation methods are the early approaches. I.e. (segmenting each compartment into one spot and its background area).Intensity Extraction: The core component extraction in microarray processing is the intensity extraction step. Expressions of gene values can be calculated based on red and green foreground intensity pairs and background intensities.

In this paper we are going to propose a new method by Optimized K-means clustering for homogeneously segmentation of microarray images. The paper is organized as follows: Section II. Presents conventional clustering algorithm, Section III. Limitations of conventional clustering algorithms presents, IV. OKM Clustering Algorithm, V. Section presents Experimental results, and finally Section VI. Report's conclusions.

II. CONVENTIONALCLUSTERING ALGORITHMS

A. K-Means Clustering Algorithm:

K-means is one of the efficient methods in clustering introduced by Hartigan in 1979 [6]. This method is applied to segment the microarray image in recent years. The main idea behind the k-means clustering algorithm is to group the pixels into clusters. Data which belongs to a definite cluster could not be included in another cluster. The objective is to minimize the sum of squares of the distances between the clusters. However, for segmenting the microarray image using k-means clustering algorithm is described as follows:

Algorithm K-means(x, n, c) Experimental Input:

N: number of pixels to be clustered;

x={x_1, x2, x3…x_N}: pixels of microarray image c = {c_1, c2, c3… cj}: clusters respectively. Here we group the pixels into two clusters,

foreground and background, j=2.
Simulated Output:
cl: cluster of pixels
Begin
Step 1: cluster cancroids are initialized.
Step 2: compute the closest cluster for each pixel and classify it to that cluster.

$$\Delta_{ij} = \| x_i - c_j \|. \quad arg\ min \sum_{i=1}^{N} \sum_{j=1}^{C} \Delta_{ij}^{2} \qquad (1)$$

Step 3:New centroids of a cluster is calculated by the following

$$c_j = \frac{1}{N\ j} \sum x_i \text{ where } x_i \text{ belongs to } c_j . \qquad (2)$$

Step 4: Repeat steps 2-3 till the sum of squares given in equation is minimized.
End.

B. Fuzzy C-Means Clustering Algorithm:

In dealing with data containing uncertainty, the Fuzzy theory has been recently used. However for analysis of DNA microarrays fuzzy clustering approaches as been taken. Fuzzy c-means is a basic Fuzzy clustering method originally introduced by Bezdek in 1981 c-means means method aims each pixel may belong to more than one cluster. So the goal is to find the membership values of pixels belonging to each cluster. The fuzzy c-means algorithm is an iterative optimization that minimizes the cost function. However, for segmenting the microarray image using Fuzzy C-means clustering algorithm is described as follows:

Algorithm Fuzzy C-Means(x, n, c, m)
Experimental Input:
N=number of pixels to be clustered;
x = {x_1, x_2... x_N}: pixels of microarray image;
c=2: foreground and background clusters;
m=2: the fuzziness parameter;
Simulated Output:
U: membership values of pixels and segmented Image
Begin
Step_1: Initialize the membership matrix u_{ij} is a value in (0, 1) and the fuzziness parameter m (m=2).

$$\sum_{j=1}^{c} u_{ij} = 1 \qquad -(3)$$

For all i= 1, 2, .N, where c is the number of clusters and N is the number of pixels in microarray image.

Step_2: Compute the Centroid values for each cluster c_j.

$$F = \sum_{j=1}^{N} \sum_{i=1}^{c} u_{ij}^{m} \| x_j - c_i \|^2 \quad --- \quad (4)$$

where u_{ij} represents the membership of pixel x_j in the i^{th} cluster and m is the fuzziness parameter.
Step_3: Compute the updated membership values u_{ij} belonging to clusters for each pixel and cluster cancroids according to the given formula.

$$u_{ij} = \frac{1}{\sum_{k=1}^{c} \left(\frac{\|x_j - v_i\|}{\|x_j - v_k\|} \right)^{2/(m-1)}},$$

and

$$v_i = \frac{\sum_{j=1}^{N} u_{ij}^{m} x_j}{\sum_{j=1}^{N} u_{ij}^{m}}. \qquad (5)$$

Step_4: Repeat steps 2-3 until the cost function is minimized.
End.

C. Moving K-Means Clustering Algorithm:

The Moving K-means clustering algorithm is the modified version of K-means proposed in [7]. It introduces the concept of fitness to ensure that each cluster should have a significant number of members and final fitness values before the new position of cluster is calculated. The Moving K-means clustering algorithm for classification of remote sensing image is summarized as follows:

Algorithm Moving K-means(x, n, c)
Experimental Input:
N: number of pixels to be clustered;
x={x_1, x2, x3… xN}: pixels of remote sensing image.
c = {c_1, c2, c3… cj}: clusters respectively.
Simulated Output:
cl: cluster of pixels
Begin
Step 1: cluster centroids are initialized
Step 2: compute the closest cluster for each pixel and classify it to that cluster,

$$\Delta_{ij} = \| x_i - c_j \|. \quad arg\ min \sum_{i=1}^{N} \sum_{j=1}^{C} \Delta_{ij}^{2} \qquad (6)$$

Step 3: The fitness for each cluster is calculated using

$$f(c_k) = \sum_{t \in c_k} (\| x_t - c_k \|)^2 \qquad (7)$$

All centers must satisfy the following condition:

$$f(c_s) \geq \alpha_a f(c_l) \qquad (8)$$

where α_a is small constant value initially with value in range 0< α_a <1/3, c_s and c_l are the centers that have the smallest and the largest fitness values. If (5) is not fulfilled, the members of c_l are assigned as members
Of c_s, while the rest are maintained as the members of c_l. The positions of c_s and c_l are recalculated according to:

$$C_s = 1/n_{cs} \Big(\sum_{t \in c_s} x_t \Big) \qquad (9)$$

$$C_l = 1/n_{cl} \Big(\sum_{t \in c_l} x_t \Big) \qquad (10)$$

The value of α_a is then updated according to:

$$\alpha_a = \alpha_a - \alpha_a / n_c \qquad (11)$$

The above process are repeated until (5) is fulfilled. Next all data are reassigned to their nearst center and the new center positions are recalculated using (3).
Step 4: The iteration process is repeated until the following condition is satisfied.

$$f(c_s) \geq \alpha_a f(c_l) \qquad (12)$$

End

D. Fuzzy Moving K-Means Clustering Algorithm:

In the Fuzzy Moving K-means clustering algorithm [9], the membership function is used in addition to the Euclidian distance to control the assignment of the members to the proper center. The algorithm minimizes the sensitivity to the noisy data by updating the moving member function. It is not obligatory for the members of the center with the largest fitness value to follow the center with the smallest fitness value. The Fuzzy Moving K-means clustering algorithm is summarized as follows:

Experimental Input:

N: number of pixels to be clustered;

$x=\{x_1,x_2,x_3,\ldots,x_N\}$: pixels of remote sensing image

$c=\{c_1,c_2,c_3,\ldots,c_j\}$: clusters respectively

m=2: the fuzziness parameter;

Simulated Output:

u: membership values of pixels and clustered Image

Begin

Step_1: Initialize the membership matrix u_{ij} is a value in (0,1) and the fuzziness parameter m (m=2). The sum of all membership values of a pixel belonging to clusters should satisfy the constraint expressed in the following.

$$\sum_{j=1}^{c} u_{ij}=1 \qquad (13)$$

for all i= 1,2,…….N, where c (=2) is the number of clusters and N is the number of pixels in remote sensing image.

Step_2: Compute the centroid values for each cluster c_j.

$$F=\sum_{j=1}^{N}\sum_{i=1}^{c} u_{ij}^{m}\,||x_j-c_i||^2 \qquad (14)$$

where u_{ij} represents the membership of pixel x_j in the i^{th} cluster and m is the fuzziness parameter.

Step 3: The fitness for each cluster is calculated using

$$f(c_k)=\sum_{t\in c_k}(||x_t-c_k||)^2 \qquad (15)$$

All centers must satisfy the following condition:

$$f(c_s)\geq \alpha_a f(c_l) \text{ and } m(c_{sk})>m(c_{lk}) \qquad (16)$$

where α_a is small constant value initially with value in range $0<\alpha_a<1/3$, c_s and c_l are the centers that have the smallest and the largest fitness values, $m(c_{sk})$ is the membership value of point k according to the smallest centre and $m(c_{lk})$ is the membership value of point k according to the largest centre. If (5) is not fulfilled, the members of c_l are assigned as members of c_s, while the rest are maintained as the members of c_l. The positions of c_s and c_l are recalculated according to:

$$C_s=1/n_{cs}\left(\sum_{t\in c_s}x_t\right) \qquad (17)$$

$$C_l=1/n_{cl}\left(\sum_{t\in c_l}x_t\right) \qquad (18)$$

The value of α_a is then updated according to:

$$\alpha_a=\alpha_a-\alpha_a/n_c \qquad (19)$$

The above process are repeated until (5) is fulfilled. Next all data are reassigned to their nearst center and the new center positions are recalculated using (3). Compute the updated membership values u_{ij} belonging to clusters for each pixel according to given formula

$$u_{ij}=\frac{1}{\sum_{k=1}^{c}\left(\frac{||x_j-v_i||}{||x_j-v_k||}\right)^{2/(m-1)}},$$

and

$$v_i=\frac{\sum_{j=1}^{N}u_{ij}^{m}x_j}{\sum_{j=1}^{N}u_{ij}^{m}}. \qquad (20)$$

Step 4: The iteration process is repeated until the following condition is satisfied.

$$f(c_s)\geq\alpha_a f(c_l) \quad \text{and} \quad m(c_{sk})>m(c_{lk}) \qquad (21)$$

III. LIMITATIONS OF CONVENTIONAL CLUSTERING ALGORITHMS

In clustering area, one of the most important and widely used algorithm in computer vision as a form of image segmentation is K-means clustering algorithm. K−means (KM) algorithm is numerical, unsupervised, non-deterministic and iterative conventional method which is familiar for simple implementation. However, the k-mean clustering algorithm has many weaknesses which are as follows:

1. The number of clusters K must be determined before the algorithm is executed and it is time consuming process.
2. The algorithm is sensitive to initial conditions .Unfortunately, without proper initialization process, in some cases, the cluster centers are trapped at local minima, leading to them to lose the chance to be updated in the next iteration.
3. Poor pixel assignment could occur if the pixel with the same minimum Euclidean distance to two or more adjacent clusters. And it may be assigned to the higher variance cluster leading to dead center problems.

To overcome the aforementioned problems, the soft membership based called the Fuzzy C- Means (FCM) clustering algorithm is proposed. The FCM algorithm is an iterative unsupervised clustering algorithm. In fuzzy C-means each pixel has simultaneously belong to a degree of clusters rather than completely belongs to one cluster called member ship and distributes membership values in normalized fashion. The Fuzzy C-means clustering algorithm has some of the weaknesses which are as follows:

1. It becomes sensitive to outliers and could not homogenously segment the images.
2. However, it may also converge to local optimum location.
3. Fuzzy C-means algorithm is unsuitable for the images corrupted by impulse noises such as salt and pepper noise.

To overcome the aforementioned problems, the fitness concept has been introduced in the moving K−means (MKM) algorithm. The MKM algorithm has the capability to overcome the three basic problems algorithm which minimizes dead centers and center redundancy problems as well as indirectly reducing the effect of trapped center at local minima problems faced so far.

The Moving K-means algorithm has the following drawbacks:

1. The Moving K-means algorithm is sensitive to noise.
2. For some cases of Moving k-means, the clusters or centers are not located in the middle or centroid of a group of data, leading to imprecise results.
3. To ensure all the clusters are active during the updating process the cluster with highest fitness value is forced to share its members with the cluster of lowest fitness value.

One of the obligations of MKM algorithm is overcome in AMKM algorithm, in ADMKM instead of moving the members of the center with the largest fitness Value to become a member of the center with the smallest fitness value, AMKM by simply assigning the members of the center with the largest fitness value if to the nearest cluster depending on the minimum Euclidean distance. The fuzzy concept was also introduced into the AMKM algorithm. The modified version of AMKM algorithm is adaptive fuzzy moving K−means (AFMKM) algorithm. However, they are also facing the same of the drawbacks; these algorithms failing to significantly update the lowest fitness cluster during the iteration and are also sensitive to initial parameters' values.

To overcome the aforementioned problems, enhanced moving K−means (EMKM) algorithm. In this EMKM algorithm, highest fitness cluster which is within the range will be assigned to the nearest neighboring cluster. Likewise, lowest fitness cluster obtains the members of the nearest neighboring cluster which lie outside of the range. However, the EMKM algorithm is less sensitive to the initial parameters. The fitness condition of the conventional MKM and its modified algorithms could not differentiate the dead centre and the cluster with zero variance during the process which results in a poor distribution of data. The pixel with equal distance to two or more adjacent clusters could be assigned to the higher variance cluster and the lower variance cluster will not be trained in the learning process which leading to hard membership problems. In such a situation the algorithms could fail to homogenously segment an image.

So in this article we proposed the optimized K−means (OKM) to overcome those weaknesses and homogenously segment the image. Optimized K-means algorithm is used to avoid dead centre and trapped centre at local minima which leads to producing better results and more homogenous segmented images

IV. PROPOSED OPTIMIZED K-MEANS ALGORITHM

In this paper, the OKM algorithm has been introduced as the modified version of the conventional K−means (KM) algorithm. The OKM algorithm fully concentrates on differentiating between the dead centers and cluster with similar intensity pixels. The pixel with the same Euclidian distance to two or more adjacent clusters is initially assigned to the dead centre and in upcoming next iteration it is assigned to the cluster with lower variance cluster, till up to no dead centre could be encountered. OKM Algorithm designed to ensure both types of centers or clusters are continuously updated in every iteration till up to the of pixels assigned to the fitting clusters. In the starting stage of the OKM algorithm, based on Euclidean distance we are going to assign all pixels to the adjacent cluster. The conflict pixels having the same Euclidean distance, the grey intensity of member are to be stored in ascending order according to their distance from the cluster with the highest fitness value and we assign the name of the array as E_r. Furthermore, the clusters are also stored in ascending order based on fitness value F_q.

As the literature study we observed one of the main problems in K-means algorithm cannot differentiate between clusters with similar intensity and the clusters without any members. In order to differentiate those clusters. In the proposed OKM algorithm, if we observe empty cluster and zero variance cluster, these clusters are sorted again in the ascending order according to the number of members in those clusters we named this array as *Hw*. Now we need to compare and map both data sets the *Er* and *Hw*. On the other hand, for the case where no dead centre is found, the pixels with the same Euclidean distance to two or more nearby clusters are directly assigned to the lowest fitness value cluster.

From the mentioned description, the implementation of the OKM clustering algorithm could be outlined as follows:

1. Initialize the all the cluster centre value and α, and let iteration $t = 0$. (Note: α is the constant and its value within range $0 < \alpha \leq 1$).
2. Based on Euclidean distance, assigning all pixels to the nearby clusters. Except conflicting pixels.
3. For starting cluster we have measure Mean Square Error value from the following equation (Note: this step is only implemented for iteration $t = 0$).
   $MSE = \frac{1}{n}\sum_{j=1}^{k}\sum_{i\epsilon c_j}\left\|p_{i(x,y)-}c_j\right\|^2$ ---- (22)
   Where $pi(x, y)$ is the i−th pixel with the coordinate (x, y) to be segmented and cj is the j−th centre or cluster and n is the no of pixels in the image.
4. For all the clusters we have to calculate fitness value based on following equation and we have to find cluster with high fitness value.
   $f(c_j) = \sum_{i\in c_j}\left\|p_{j(x,y)-c_j}\right\|^2$ --------- (23)
5. For the pixels having the same Euclidean distance to two or three adjacent clusters, sort the grey intensity of these pixels in the ascending order according to their distance from c_l and denote the sorting array as *Er*, where $r = 1,2,3\ldots(K\text{–}1)$.
6. Find the empty cluster
   i) If cluster without members is found
      a. Sot all clusters in the ascending order according to their fitness values and name the sorting array as*Fq*, where $q = 1, 2, 3 \ldots k$.
      b. for all clusters obtained in step 6i)(a), sort these clusters in ascending order according to the number of pixels or members in the clusters and denote the sorting array as *Hw*, where $w = 1, 2, 3\ldots.z$ (z is the total number of empty and zero variance clusters)
      c. Begin with assign the pixels with grey intensity of *Eb* to *Hb* where $b = 1$ and continue until the value of b in *Hb* equals to z or the value of b in *Eb* equals to $(k\text{–}1)$.
   ii) If cluster with member is found
      a. We begin to assign the pixels with grey intensity *Eb* to the clusters with the lowest fitness value among their adjacent clusters, where $b = r = 1, 2..k\text{–}1$
7. Increase iteration by $t = t + 1$, and update the centre positions, and measure the Mean Square Error

value using following Eqs

$$c_j = \frac{1}{n_j}\sum_{i\in c_j} c_j(x,y) \text{ --------}(24)$$

Where, $n\,j$ is the number of pixels in the j−th cluster

$$MSE = \frac{1}{n}\sum_{j=1}^{k}\sum_{i\in c_j}\left\|p_{i(x,y)-}c_j\right\|^2 - -(25) \quad .$$

8. Repeat steps 2 to 7 (except step 3) until the condition $\left\|MSE^{(t+1)} - MSE^{t}\right\|$<α---- (26)
Is fulfilled. Where 0 <α≤1. The typical value of α to obtain a good segmentation performance should be close to 0.

IV. EXPERIMENTAL RESULTS

The proposed five different clustering algorithms are performed on a sample microarray slide that has 48 blocks, each block consisting of 110 spots. A sample block has been chosen and 36 spots of the block have been cropped for simplicity. The sample image is a 198*196 pixel (gray scale) image that consists of a total of 38808 pixels.

The segmentation step implemented separately by four clustering methods, K-means, Moving K-Means, Fuzzy c-means and Fuzzy Moving K-means Optimized K-means for homogeneously segment the image respectively. These methods are implemented in such a way that the grayscale intensity value of all the pixels in the image are grouped into two clusters. The segmented microarray images after each clustering methods have been performed are shown in figure 1.

Table 1: The number of pixels clustered as spots and background

Method	Spots	Background
K-means	11986	16822
Fuzzy K-means	12774	17034
Moving K -means	13163	14645
Fuzzy Moving K-means	14019	14789
Optimized K-means	15019	15789

Quantitative analysis is a numerically oriented procedure to figure out the performance of algorithms without any human error. The Mean Square Error (MSE) 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

$$MSE = \frac{1}{N}\sum_{j=1}^{k}\sum_{i\in c_j}||v_i - c_j||^2 \qquad (27)$$

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 2 shows the quantitative evaluations of four clustering algorithms after segmenting the microarray image. The results confirm that Optimized K-means algorithm produces the lowest MSE value for segmented microarray image.

Table 2: MSE values of four segmented Images

Method	MSE Values
K-means	324.781
Fuzzy C-means	276.392
Moving K-means	187.327
Fuzzy Moving K-means	136.322
Optimized K-means	102.125

V. CONCLUSIONS

This paper has presented conventional clustering algorithms like K-means, fuzzy C-means, Moving K-means, and Fuzzy Moving K-means for the segmentation of microarray image. All these Clustering algorithms are having some advantages and Disadvantages. The OKM algorithm that can homogenously segment an image into regions of interest and OKM effectively reduce the problems like dead centers and trapped centers at local minimum.

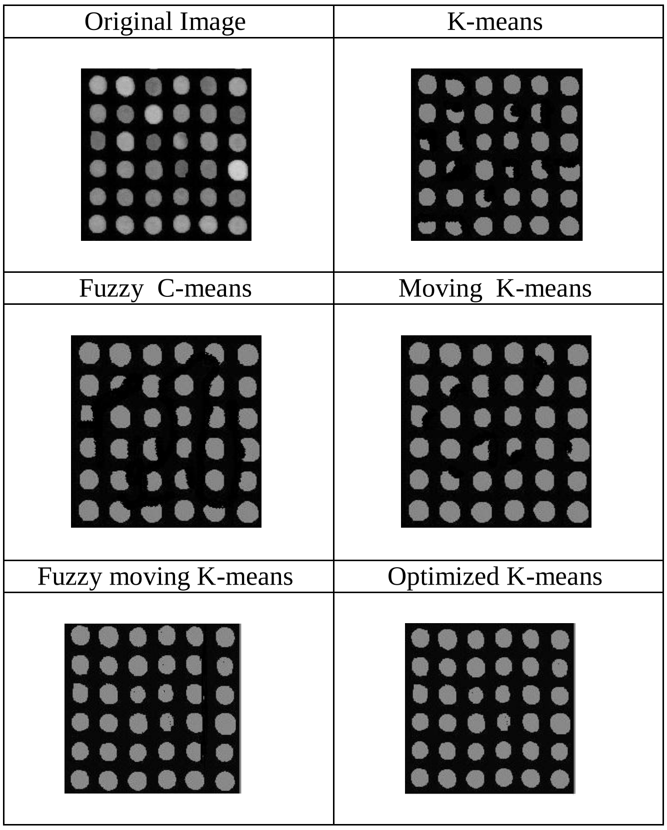

Fig1: Image segmentation results.

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