International Journal of Electrical and Computer Engineering (IJECE)
Vol. 10, No. 1, February2020, pp. 746~756
ISSN: 2088-8708, DOI: 10.11591/ijece.v10i1.pp746-756

Microarray spot partitioning by autonomously organising maps through contour model

Karthik S. A.[1], Manjunath S. S.[2]
[1]Department of Information Science and Engineering, Dayananda Sagar Academy of Technology and Management, India
[2]Department of Computer Science and Engineering, ATME, India

Article Info

Article history:

Received May 12, 2019
Revised Sep 29, 2019
Accepted Oct 5, 2019

Keywords:

AOM
C-V model
Level set
Microarray
Spot partition

ABSTRACT

In cDNA microarray image analysis, classification of pixels as forefront area and the area covered by background is very challenging. In microarray experimentation, identifying forefront area of desired spots is nothing but computation of forefront pixels concentration, area covered by spot and shape of the spots. In this piece of writing, an innovative way for spot partitioning of microarray images using autonomously organizing maps (AOM) method through C-V model has been proposed. Concept of neural networks has been incorpated to train and to test microarray spots.In a trained AOM the comprehensive information arising from the prototypes of created neurons are clearly integrated to decide whether to get smaller or get bigger of contour. During the process of optimization, this is done in an iterative manner. Next using C-V model, inside curve area of trained spot is compared with test spot finally curve fitting is done.The presented model can handle spots with variations in terms of shape and quality of the spots and meanwhile it is robust to the noise. From the review of experimental work, presented approach is accurate over the approaches like C-means by fuzzy, Morphology sectionalization.

Corresponding Author:

Karthik S. A.,
Department of Information Science and Engineering,
Dayananda Sagar Academy of Technology and Management,
KanakpuraRoad, Udaypur, Bangalore- 560082, Karnataka, India.
Email: karthiksa1990@gmail.com

1. INTRODUCTION

In the domain of bioinformatics cDNA microarray is fastest developing technology which helps researchers to quantify the behavior of genes (in terms of thousands)at the same time. Microarray substrate consists of tiny spots which expresses some unique information about the genes [1]. Substrate preparation is done by collecting mRNA samples the purpose of collecting mRNA is to identify which specific genes are actively involved in giving gene expression. Next, collected mRNA sample will undergo a process called hybridization where sample get transcript into cDNA. Further, samples prepared by the mentioned process were plotted on microarray substrate. At last, entire substrate is scanned at a specific laser wavelength which results in a microarray image.

By matching normal spots with diseased spot one can judge which gene is affected and can develop a drug for curing diseased gene [2]. Entire microarray analysis involves three major steps like array localization, separating spot foreground from background, quality assesment [3]. Array localization involves in identifying exact location of gene spot in sub grid. Once location of the spot is identified in a sub grid next task is to separating a spot from its background such separation is popularly known as spot separation. Last step is to computing unique spot intensity values of a substrate.

Image segmentation is defined as the process of dividing an image into constituent regions or objects. In the context of DNA microarray images, segmentation's goal is to divide each grid cell into regions

corresponding to the spot (foreground) and background. Segmentation is typically performed after the microarray image has undergone a gridding step and it has been divided into cells (also known as sub-grids). After segmentation, gene expression levels are estimated from the foreground area. A number of factors make the segmentation of microarray images a challenging task. To name a few, the background is typically contaminated by noise. Spots' shapes and sizes might differ within an image from one spot to another. The intensity of a spot is not necessarily uniform. Also since the hybridization process is not homogeneous the spot regions could be broken. Moreover, the quality of DNA microarray images might vary.

The background noise and variation in the shape of the spots can be clearly seen in the picture. Figure 1 exhibits some sample spots of different DNA microarray images. As can be seen there is a wide range of variations in terms of shape and quality of the spots. Seeing that, segmentation methodologies typically necessitates manual interference to give the requisite parameters or to exact their results. Conversely, there is a less scope of automation that can extensively influences biological interpretations. In fact, it has been shown that the choice of the segmentation method has significant effects on the provision of the outcome of an experiment.

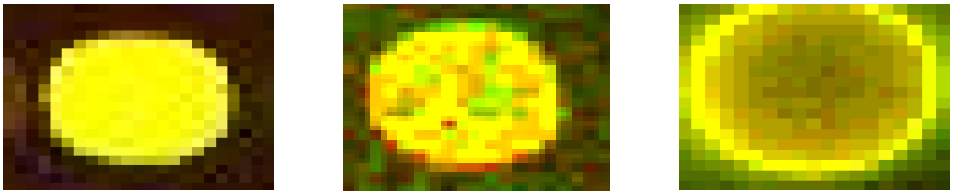

Figure 1. Typical microarray spots

Hence, for the perfect categorization of gene expression and to generalize spot's eminence measures there is a great demand for the robostic approach. From the aforementioned stages, one can conclude spot partitioning is one of the most challenging task.

A few years back, numerous mercantile packages and investigative methods have been proposed for the segmentation of images. Existing segmentation [4-8] (partition of the forefront area from the entire image) methodologies are categorised as ring fixed segmentation, adaptation ring segmentation, morphological adaptation segmentation. In GenePix versatile ring division method is utilized. As per the approach, the radius of all spot is not regular but fine tuned independently for every spot. Nevertheless, a round spot mask yields a worst fit to unpredictable spots as the methodology restricts the shape of the regions as circular. In ScanAlyze [9-12], a fixed ring segmentation method implemented by means of the hypothesis that areas are assumed to be globular with a preset radius. Spot-on also uses the same principle of fixed circle segmentation.

The SPOT Software incorporates the methods [12] such as watershed and seeded region growing Advanced image handling techniques used in the domain of microarray consist of the morphological adaptation segmentation. This approach does not dependent on any postulation about the dimension and the contour of the spot. The flaw of both watershed and seeded region growing approaches are, they demand the precise condition of the preliminary points or the seeds. The seeded region growing approach [13] is further flexible and adaptable to diverse contours and dimensions of the spots but it is quite sensitive to noise.Parameters such as starting value and porch value are selected by considered spot. Approach works on low intensity values of spots, improper shaped spots also. Abrupt pixel values do not affects execution of proposed approach.

Most important difficulty of histogram approaches is that quantification is unsteady after a great intention mask is situate to variation. Active contour methodologies has been discussed in [14, 15], where a preliminary contour is to be found on the spot-image and a contour bend is produced for the job of outline the spot boundaries.Mixture model methods [16, 17] are incorporated for the job of partitioning microarray images. On the other hand, these methods are dependent on intensity and do not think about the reliable among adjoining pixels. They rely on assumption of normality for the application to the segmentation problem (parametric assumption of normal distribution). Other scholars initiated much relevant procedures for the process; to fusion sculpt analysis [17] and deformable models [18]. In the mixture model analysis, the most important negative aspect is the detection of intensity near to its spot background. Approaches such as K-means , Fuzzy C means , Expectation-Maximization [19-22] etc., have been used by several scholars. K-means reflect on a few narrow features, like noise. Also, the partitioned forefront and surroundings section need not be linked in this, technique but actually the forefront and backdrop are associated regions. The most important negative aspect is, they not get used to fit to irregular base clusters and fails to utilize all

the available prior knowledge about the data.Offset vector field and expectation maximization algorithm describes a unique approach to suppress unwanted pixels in genes. Using maximization algorithm separation of background done. Experimental results reveals that proposed approach is attractive and yields very promising performance.Graph based methods [23], describes a way for classification of pixels. Author conveys that spot segmentation of microarray is a challenging task and proposed approach is automated. Described approach works on irregular spot shape and size which results in high accuracy of approach.

Self-motivated curvature approach [24] projects a few ideas to partiton an image by ignoring edge picture values. Image boundariesare undoubtedly rectified with slight changes. Level set and C-V approach [25, 26] has illustrated an innovative way to separate image using level set scaffold. Boundary values can help to find out precise location of intention shape and resolves dilemma of border line leakage Descriptive instances of the work carried out signifies the helpfulness ofthe method. A new automated approach [27] for segmenting microarray images describes a way for achieving optimization through genetically based algorithm. Proposed approach is advantageous over noise and results are excellent when they are tested during worst case. Illustrates [28] innovative section support method for image sectionalisation that is capable to resolve concentration in homogeneity. Primarily confined intensity based grouping criterion has been functional then incorporated with comprehensive criterion for image. Ultimately reducing of energy is conceded out by L-S model. Anda modified version [29] of clustering algorithm to suppress unwanted pixel information from images. Initially, quality of the image improved using enhancement approach then using variance between class of spots method is applied to address the spot. Finally, clustering algorithm is applied to spots. A novel way [30] to segment the image by reducing level of noise is achieved .Initially the proposed grow-cut algorithm applied to every spot to classify from its background. To assess the novelty, the proposed approach is implemented through multithreded CPU and GPU. Proposed approach [31] is combined some of the popular traditional algorithm like canny, morphology, FCM. By experimental results the proposed algorithm is fast enough to segment the spots. To classify the spot as forefront part and background obtained image is pre processed followed by separation of spot from forefront part. Next, unique expression is computed [32] and data is normalized. Finally obtained results are optimized.

In micro image analysis, it is necessary to do an image partition as a forefront area and background region. A major problem that affects microarray image partitioning is an irregular distribution of intensity values. Sometimes these intensity values appear to reside in the background or it may be in the forefront area of the spot. This leads to confusion whether a pixel belongs to the forefront area of spot or background region. This paper discusses a novel method to solve the misclassification of pixels as forefront pixels or background pixels. The whole editorial composition framed as : division 2 describes innovative way for spot partitioning based on AOM-CV model. Division 3 describes the experimental values of some test spots. Presented work is eventually concluded at the last.

2. AUTONOUMOUSLY ORGANISING MAPS THROUGH CHAN VESE(AOM-CV) METHODOLOGY

In the present work, a novel unsupervised autonomously organizing map based on Chan Vese (AOM-CV) is proposed. This model is based on a group of trained autonomously organizing neurons to store intensity values of the image. AOM-CV model employs AOM as a tool to explore the intensity distribution of a spot and joins the prototypes of learned AOMs to control the growth of contour. These prototypes are employed to estimate intensity distribution and to guide the growth of contour. The general idea of the methodology is shown in Figure 2. Spots are captured from typical databases such as GEO-DB [33]. This work contributes.

– Formulation of unsupervised active contour model
– Guide for contour growth
– Presents a well experimental work in terms of accuracy and robustness

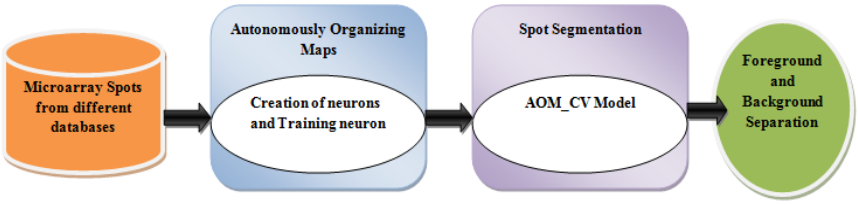

Figure 2.Overview of AOM-CV approach

2.1. Formulation of activecontour models

The C-V model and M-S model [34, 35] are the well known mathematical models for segmentation based on region. C-V model is based on reducing approximation function, developed in such a way that it's minimum reached by close approximation of exact edges of different regions. The energy function is given by:

$$E_{CV\,(C_r)} = \mu \cdot \text{len}(C_r) + \nu \cdot \text{Area}(C_r) + \lambda^{+} \int_{C_{rin}} \left(Sp(x) - c^{+}(C_r)\right)^2 dx + \lambda^{-} \int_{C_{rout}} \left(Sp(x) - c^{-}(C_r)\right)^2 dx \quad (1)$$

Figure 3 shows representation contour, where, C_r is the contour and Sp(x) is a microarray spot such that Sp(x)∈ℝ.Sp(x)is the grey level value of the spot denoted by location of the picture element in microarray spot denoted by Ω. Parameter of regularization μ≥0 helps in contour smoothness inside and outside area of the microarray spot is denoted by C_{rin} and C_{rout} of C_r. ν≥0 another penalizing parameter for huge area offorefront region of the spot. One can write:

$$c^{+}(C_r) = \text{Expectation}(Sp(x) | x \in C_{rin})$$
$$c^{-}(C_r) = \text{Expectation}(Sp(x) | x \in C_{rin}) \quad (2)$$

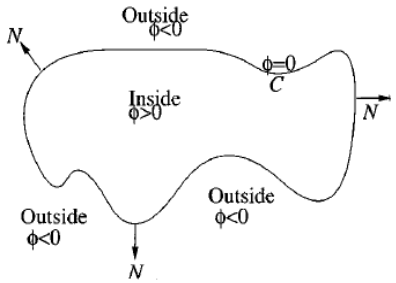

Figure 3. Representation of contour

In (2) represents expected values of intensities of area inside and outside the curve.λ^{+} and $\lambda^{-} \geq 0$ which control the influence of the two image energy terms

$$\int_{C_{rin}} ((Sp(x) - c^{+}(C_r))^2) dx \text{ And} \int_{C_{rout}} ((Sp(x) - c^{+}(C_r))^2) dx \quad (3)$$

In a formulation of level set C_r is expressed as null set function Ø:Ω→ℝ:C_r={x∈Ω:Ø(x)=0}. Area inside and outside the spot area of contour also expressed as

$$C_{rin} = \{x \in \Omega: Ø(x) > 0\}$$
$$C_{rout=}\{x \in \Omega: Ø(x) < 0\} \quad (4)$$

Distance function to express the contour Ø(x) as Euclidian distance is given by:

$$Ø(x) = \begin{cases} \text{dist}(x, c) & x \in C_{rin} \\ 0 & x \in Cr \\ -\text{dist}(x, c) & x \in C_{rout} \end{cases} \quad (5)$$

In level set formulation of C-V model, the reliance of $C^{+}(C_r)$ and $C^{-}(C_r)$ onØdepends on minimization of eq(1) which is done by applying gradient decent leading to following PDE.

$$\frac{\partial Ø}{\partial t} = \delta(Ø)\left[\mu\nabla \cdot \left(\nabla Ø / \|\nabla Ø\|\right) - \nu - \lambda^{+}((Sp - c^{+}(Ø))^2) + \lambda^{-}((Sp - c^{+}(Ø))^2)\right] \quad (6)$$

||.|| is norm of euclidian and δ(.) is the Dirac generalized function. To keep the level set function smooth, μ is used. To control the growing rate of the evolving contour term ν is used. λ^{+} and λ^{-} can be generalized as inner and outer forces that force the contour toward the definite spot's border line.

2.2. Spot segmentation using AOM and Chan-Vese model (AOM-CV model)

In AOM-CV model involves two stages namely, training and testing stage. In the training stage, a set of points are extracted from an edge detection procedure from the given image are used as the input vector. The weighting vector is assigned initially and then updated. The training stage is followed by the testing stage where the edge points are controlled according to a PDE. Finally, the model is implemented by an algorithm.

2.2.1. Training session

During a training session by selecting a appropriate number of neurons [35, 36] and topology [37] for AOM the intensity $SP_{in\_tr}(x_t)$ of arbitrarily chosen pixel x_t of training image taken as AOM input at time t=0, 1,…….$t_{max\_tr.}$ Let t_{max_tr}be the iterating value for training of AOM. Subsequently, neurons are autonomously arranged to maintain its topological structure. Every neuron n is associated to the weigh vector w_n^+ of D dimension once initialization is completed weight w_n^+ rule can be written as:

$$w_n^+(t+1) = w_n(t) + \eta(t)h_{bn}(t)\left[SP_{int_{tr}}(x_t) - w_n(t)\right] \quad (7)$$

Rate of learning is symbolically represented by η(t), the feasible matching unit (FMU) of number of neuron b around kernel neighbourhood denoted as $h_{bn}(t)$. To optimize the weights $w_n^+(t)$, the term η(t) and $h_{bn}(t)$ must be developed as function of time decreasing. Once training session of neuron is completed, one can approximately map pixel intensities distribution to weights of FMU. Therefore choice of learning rate is

$$\eta(t) = \eta_0(t)\exp\left(\frac{-t}{\tau_n}\right) \quad (8)$$

$\eta_0$is the initial rate of learning $\eta_0 \geq 0$ and $\tau_n > 0$ is a time constant where as $h_{bn}(t)$ chooses a function of Gaussian centred on neurons assume the form

$$h_{bn}(t) = exp\left(-\left\|\frac{r_b - r_n}{2r^2(t)}\right\|^2\right) \quad (9)$$

Where r_b and $r_n \in \mathbb{R}$ are address of vectors in the neural map of output of neuron b and n. time decreasing neighbourhood of radius r(t)>0 is given by$r_o$initial neighbourhoodand τ_r

$$r(t) = r_0 exp\left(\frac{-t}{\tau_r}\right) \quad (10)$$

Thus, values of weights$w_n^+(t+1)$ are obtained in the training session.

2.2.2. Testing session

After successful completion of training session of AOM-CV model, trained network is deployed to testing phase with typical microarray spots for the curve progression of C_r. At the time of evolution of curve forefront image intensity and background intensity say $E(Sp(x) \mid x \in C_{r\_in})$ and $E(Sp(x) \mid x \in C_{r\_out})$ respectively are passed as input to the trained network. For every neuron the quantities are defined as

$$A_n^+(C_r) = |w_n - E(Sp(x)|x \in C_{rin})|$$
$$A_n^-(C_r) = |w_n - E(Sp(x)|x \in C_{rout})| \quad (11)$$

$A_n^+(C_r)$and$A_n^-(C_r)$ are the distances of subsidiary prototype w_n from mean intensity of current approximations of forefront of a spot and background of a spot and iteratively computed during testing session. Then, one can define the two sets,

$$\{w_j^+(C_r)\} = \{w_n : A_n^+(C_r) \leq A_n^-(C_r)\}$$
$$\{w_j^-(C_r)\} = \{w_n : A_n^+(C_r) > A_n^-(C_r)\} \quad (12)$$

$N^+(C_r) = |\{w_j^+(C_r)\}|andN^-(C_r) = |\{w_j^+(C_r)\}|$which are associated prototypes of set of neuron. According to their expected value of intensity such prototypes are chosen as forefront area and background area of spot. Functional of AOM C-V model is given by:

$$E_{AOM\_CV}(C_r) = \lambda^+ \int_{C_{rin}} e^+(x, C_r)dx + \lambda^- \int_{C_{rout}} e^-(x, C_r)dx \quad (13)$$

Where, image energy terms are

$$e^{+}(x, C_r) = \sum_{j=1.....N^{+}(C_r)} \left(Sp(x) - w_j^{+}(C_r)\right)^2 \quad (14)$$

$$e^{-}(x, C_r) = \sum_{j=1.....N^{-}(C_r)} \left(Sp(x) - w_j^{-}(C_r)\right)^2 \quad (15)$$

Where, parameters λ^+ , $\lambda^- \geq 0$, are the associated weights of spot energy terms. For the level set function replace C_r by Ø then

$$E_{AOM\_CV}(\emptyset) = \lambda^{+} \int_{\emptyset>0} e^{+}(x, \emptyset)dx + \lambda^{-} \int_{\emptyset<0} e^{-}(x, \emptyset)dx \quad (16)$$

The explicit reliance of e^+ and e^- on level set function Ø is based on Heaviside step function H: $\mathbb{R} \rightarrow R$

$$\text{Hev}(z) = \begin{cases} 1 & \text{if } z \geq 0 \\ 0 & \text{if } z < 0 \end{cases} \quad (17)$$

By rewriting equation of $E_{AOM\_CV(\emptyset)}$

$$E_{AOM\_CV}(\emptyset) = \lambda^{+} \int_{\Omega} e^{+}(x, \emptyset)\left(H(\emptyset(x))\right)dx + \lambda^{-} \int_{\Omega} e^{-}(x, \emptyset)(\,1 - \left(H(\emptyset(x))\right)dx \quad (18)$$

Finally contour evolution is given by

$$\frac{\partial \emptyset}{\partial t} = \delta\emptyset[\lambda^{+}e^{+} + \lambda^{-}e^{-}] \quad (19)$$

From (19), can be solved iteratively using the same smoothing and discretization techniques.

2.3. Algorithm for AOM CV implementation

Algorithm for AOM CV model implementation

1.Intialization of input parameter

Intialization of input parameters

a. Train_spot= "image.bmp" and Test_spot= "image1.bmp"
b. No. Of Iteration and Max. Iteration $t_{max_tr}$and t_{max_evol}
c. Global segmentation=σ, learning rate and radius of map η and r_o
d. Time constants, weights of energy and binary approximation of level set function: (τ_n and τ_r), (λ^+ and λ^-) and ρ

2. Neuron prototype Intialization: Training Session
   Do
3. To determine FMU of neuron to intensity $I_{int\_tr}(x_t)$ choose a pixel x_t in a domain of spot Ω :
4. Model updating using equation (7),(8),(9) and (10)
5. While(t_{max_tr}=t_{max_evol})
6. Testing Session
   Intialization of level set function in the domain of spot Ω with boundary Ω' whose subset is Ω_0
7. If($x \in \Omega_0/\Omega'$) then x=ρ
8. Else if ($x \in \Omega'$) the x=0
9. Otherwise x=-ρ
   Do
10. If $E_{AOM\ C\text{-}V}$ has been selected then
11. Calculate A_n^+ and A_n^- for every neuron
12. Grow the contour using Ø
13. Else compute w_b^+ and w_b^-
14. End if
15. Update current Ø to binary using Ø←ρ(H(Ø)-H(-Ø))
16. Stop evolution if t_{max_tr}reached.

From this algorithm, one can implement this using MATLAB.

3. EXPERIMENTAL STUDY AND OUTCOMES

In the experimental study, the rejoinder of the put forwarded approach is estimated on a variety of microarray spots. Images are captured from GEO-DB. Depending on type of spot four tupes of image are analyzed. The four spots are: typically clean spot, spot with artifacts, donut spot and noisy spot. In this work MATLAB (R2014b) is used for image analysis as it is a high performance tool. To quantify the level exact partitioning of forefront part from rear part region, superiority of the spot, various considerations such as Total image Eminence index (TIEI), coefficient of determination r^2 , concordance correlationp_c, are used.

3.1. Total image eminence index (TIEI)

To make a distinction the trained and testable spot this parameter gauge is utilized [32].In described methodology partitioned spot is alienated into correlation loss, luminance distortion and morphological contrast is specified by

$$corrloss(i,j) = \frac{st(i,j)}{st(i)\cdot st(j)} \tag{20}$$

$$Lu(i,j) = \frac{2\cdot st(i)\cdot st(j)}{(st(i))^2+(st(j))^2} \tag{21}$$

$$S_t(i,j) = \frac{2\cdot st(i,j)}{(st(i))+(st(j))} \tag{22}$$

where $\mu_i and \mu_j$ average outline of curvature and partitioned curvature, st(i) and st(j) are normal divergence of spot and partitioned spot, st(i,j) is co-variance of trained curvature and partitioned curvature. In (20) is a measure which computes the relation ship between input spot and segmented spot. Range of corrloss (i,j) is [-1, 1]. Best value for this parmeter is 1. Lu(i, j) value lies between 0 and 1 which clearly measures closeness value of luminance between spot taken as input and portioned spot. Morphological contrasts between images are measured by S_t(i, j) its range of value is between 0 and 1. Good value for Lu(i, j) and St(i, j) is 1. Ultimately Total Image Eminence index (TIEI) conveyed as [38, 39].

$$TIEI = Lu(i,j) * Con(i,j) * St(i,j) \tag{23}$$

$$TIEI(i,j) = \frac{4\cdot\mu_i\cdot\mu_j\cdot st(i,j)}{(\mu_i+\mu_j)^2+(st(i))+(st(j))} \tag{24}$$

In general choice of this parameter is [-1, 1]. As shown in Table 1 shows statistical assessment of TIEI for spot partition. When TIEI is equal to unity or almost unity signify best quality of spot,-1 signifyworstquality.

Table1. Statistical assessments of TIEI for spot partition [38, 39]

	TIEI of a range of spots		
Sp_id[16]	C-means by fuzzy	Morph_ Sectionalization	AOM-CVtechnique
Spot8	0.418	0.625	0.950
Spot9	0.440	0.351	0.483
Spot6	0.320	0.621	0.792
Spot12	0.460	0.326	0.616
Spot13	0.550	0.710	0.733

In order to make a review of segmentation process the following metrics are calculated from the simulated images:

– **The coefficient of determination r^2** indicates the strong point of the linear involvement between simulated and calculated spots, as well as, it give the proportion of the fluctuation of the calculated data [40].

$$r^2 = \frac{\sum_{i=1}^{All\ Spots}(Isegmented(i)-Iactual)^2}{\sum_{i=1}^{All\ Spots}(Iactual-\bar{I}actual)^2} \tag{25}$$

Where$I_{segmented}$ and $I_{actual}$are the mean intensity values of the calculated and simulated spots, respectively. Here*i* refers to individual cell images (*i*= 1….324), and $\bar{I}actual$ is the overall mean of the foreground of all spots in simulated image. The algorithm that sets r^2 value closest to the unity has the best performance.

– **The concordance correlation p_c** measures the agreement between simulated and calculated data and is used to evaluate the reproducibility of the proposed segmentation method [41].

$$p_c = \frac{2*SD_A*SD_B r}{{SD_A}^2+{SD_B}^2+(\mu_A-\mu_B)^2} \quad (26)$$

Where A and B are two samples, μ_A and μ_B are the mean values, SD_A and SD_B are the standard deviation of the samples. p_c value decides the performance of algorithm, higher the p_c values tells that better performance of approach. Comparision oof existing method with our technique using assessment parameters is shown in Table 2.

Table 2. Comparison of existing method with our technique using assessment parameters

Image Id	C-means by fuzzy(FCM)		Morph_ Sectionalization(MoSeg)		AOM-CV technique	
	r^2	p_c	r^2	p_c	r^2	p_c
Spot	0.8612	0.7480	0.8630	0.9190	0.98350	0.9917
Spot8	0.8721	0.6727	0.5732	0.6328	0.9721	0.9857
Spot9	0.6840	0.4900	0.7693	0.8525	0.9607	0.9801
Spot6	0.4306	0.1848	0.6829	0.7682	0.8972	0.9422
Spot12	0.8055	0.7261	0.5706	0.6356	0.8981	0.9372
Spot13	0.8107	0.6315	0.7335	0.8033	0.9938	0.9968

Figure 4 shows boxplot comparision of TIEI. Box plot of Fuzzy C-means method is comparatively short-which suggests that generally spot's total image eminence index [40-42] have a high level of agreement with each other but fails to reach closest to unity. Morpho-segmentation method box comparatively tall and interprets different imminence index value when compared to other spots. AOM-CV method box plot shows close value of imminence index to 1 which is a promising result to judge the quality of the spot. From Figure 5 and Figure 6 it is clear that there is close approximation of AOM_CV method towards the best performance of spot portioning, compared to FCM method and Moseg method. Figures 5 and 6 demonstrates our experimental work with different types of spot.

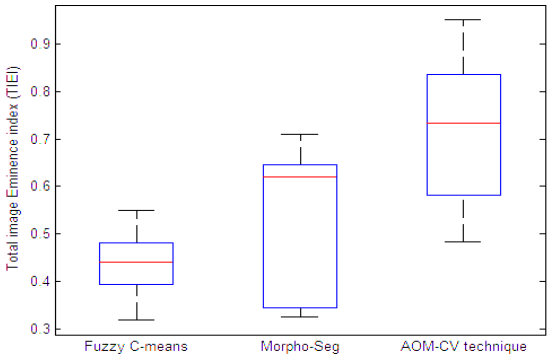

Figure 4. Box-plot comparison of TIEI

.

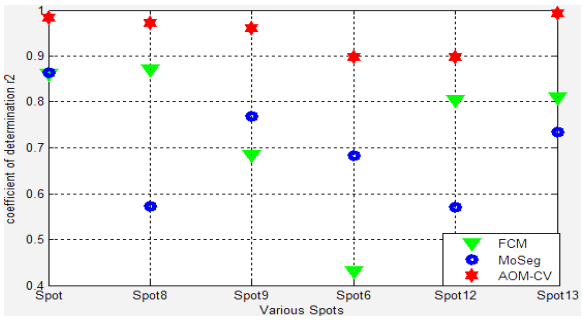

Figure 5. Comparison of r^2

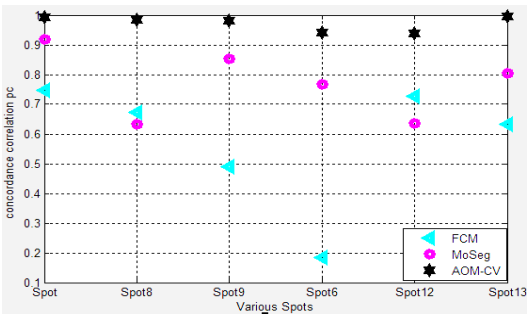

Figure 6. Comparison of p_c

Figure 7 shows experimental study on various types of spots. For the purpose of experimental study various types of spots are considered. AOM-CV approach works well in all cases including noise, donut shaped spot. Moreover it identifies contour in spots (donut spot). Finally forefront area and background region clearly differentiated.

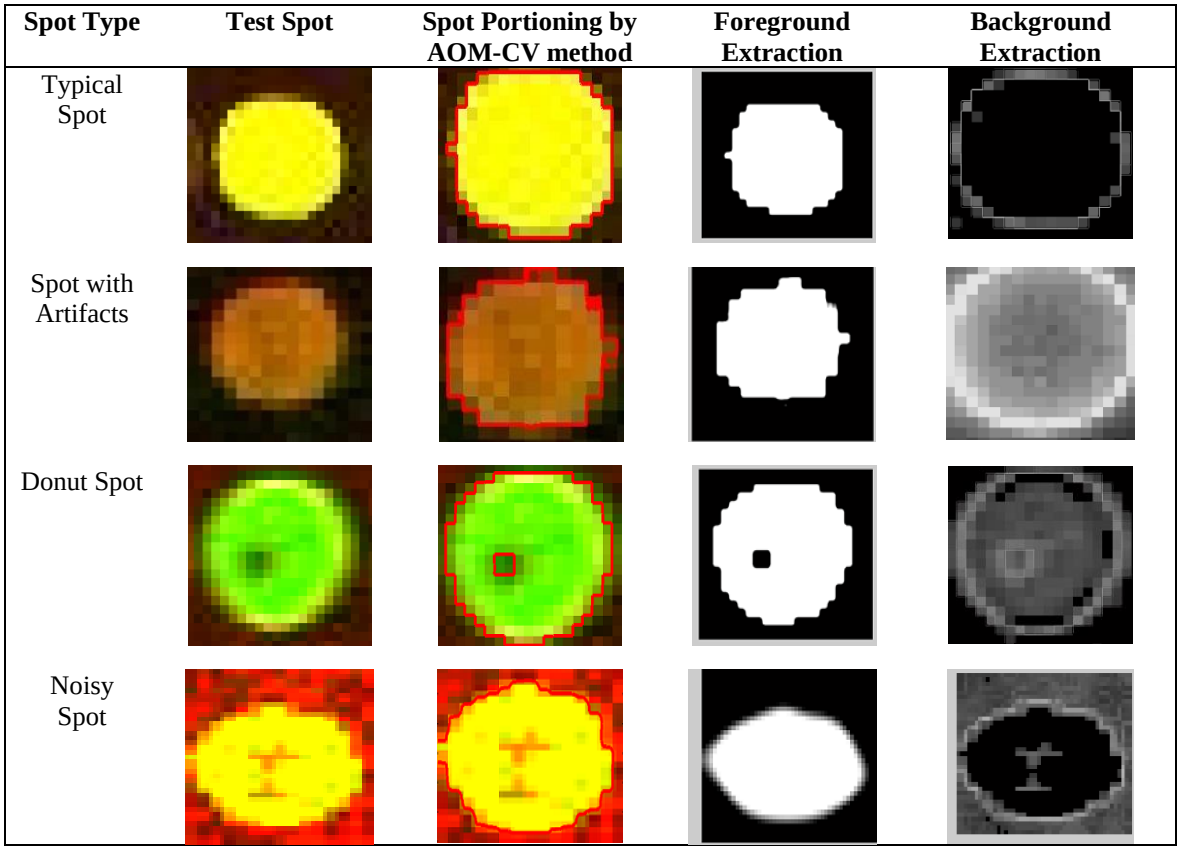

Figure 7. Experimental outcome

4. CONCLUSION

In this work, a novel method of spot portioning images is done. First, the testing of neurons and the training process of neurons is carried out using automatic organizing maps (AOM). This method considers the average intensity values inside and outside the curvature of the spot. Evolution of contour is done by edge map. From this one can easily distinguish the forefront area from the rear region. Now, this resolves the challenges created by the other methods. AOM-CV approach is also applicable images that consist of arbitrary deviated of pixels also. Through conducting tests and obtained results total procedure is dynamic, in the presence of arbitrary deviation of pixels, topological changes, and irregularity of spot and morphological changes.

REFERENCES

[1] X. Xie and G. Beni, "A Validity measure for Fuzzy Clustering,"*IEEE Transactions on Pattern Analysis, machine Intell*, vol. 13(8), pp. 841-847, 1991.
[2] "Introduction to microarray, "[Online], Available: www.cebitac.unibielefeld de/groups/brf/software/emma.
[3] Schena M, Shalon D, Davis R W and Brown P O, "Quantitative monitoring of gene expression patterns with a complementary DNA microarray,"*Science*, vol. 270(5235), pp. 467-70, 1995.
[4] Buhler J, Ideker T, &Haynor D, Dapple, "Improved techniques for finding spots on DNA microarrays," (UWCSE Tech), Washington UWTR department of computer science and Eng, University of Washington, pp. 198-207, 2000.
[5] Buckley M. J, "Spot user's guide," Sydney, Australia: *CSIRO Mathematical and Information Sciences*, 2000.
[6] Wang X, Ghosh S, Guo SW, "Quantitative quality control in microarray image processing and data acquisition,"*Nucleic Acids Research*, vol. 29(15), pp. e75, 2001.

[7] E. K. Lobenhofer, P. R. Bushel, C. A. Afshari, and H. K. Hamadeh, "Progress in the application of DNA microarrays,"*Environment Health Perspectives*, vol. 109(9), pp. 881–891, 2001.

[8] Yang YH, Buckley MI, Dudoit S, Speed TP, "Comparison of methods for image analysis on cDNA microarray data,"*Journal of Computational and Graphical Statistics*, vol. 11(1), pp. 108–36, 2002.

[9] Bozinov D, Rahnenführer J, "Unsupervised technique for robust target separation and analysis of DNA microarray spots through adaptive pixel clustering,"*Bioinformatics*, pp.747–56, 2002.

[10] Ahmed AA, Vias M, Iyer NG, Caldas C, Brenton JD, "Microarray segmentation methods significantly influence data precision," *Journal of Nucleic Acids Research*., vol. 32(5), pp. e50, 2004.

[11] M. B. Eisen, "ScanAlyze, "[Online], Available: http://rana.Stanford.EDU/software/for software and documentation.

[12] K. Blekas, N. P. Galatsanos, and I. Georgiou, "An unsupervised artifact correction approach for the analysis of DNA microarray images," in *Proc. IEEE Int. Conf. Image Process. (ICIP)*, vol. 2, pp. 165–168, 2004.

[13] Deepa J, Tessamma Thomas, "Automatic Segmentationof DNA Microarray Images using an Improved seeded region growing method,"*World Congress on Nature & Biologically Inspired Computing (NaBIC), IEEE,* 2009.

[14] T. Srinark and C. Kambhamettu, "A microarray image analysis system based on multiple snakes," *Journal Biol. Syst. Spec. Issue*, vol. 12, pp. 127–157, 2004.

[15] J. Ho and W.-L. Hwang, "Automatic microarray spot segmentation using a snake-fishermodel," *IEEE Transactions on Medical Imaging*, vol. 27(6), pp. 847–857, 2008.

[16] K. Blekas, N. Galatsanos, A. Likas, and I. E. Lagaris, "Mixture model analysis of DNA microarray images,"*IEEE Trans. Med. Imag.*, vol. 24(7), pp. 901–909, 2005.

[17] Athanasiadis E.I, *et al.*, "Complementary DNA microarray image processing based on the Fuzzy Gaussian mixture model,"*In IEEE Transaction on Information Technology in Biomedicine*, vol. 13(4), 2009.

[18] Ceccarelli M, Antoniol G, "A deformable grid-matching approach for microarray images,"*IEEE Transaction on Image Processing*, vol. 15(10), pp. 3178–88, 2006.

[19] Rahnenführer J, Bozinov D, "Hybrid clustering for microarray image analysis combining intensity and shape features,"*BMC Bioinformatics*, pp. 1–11,2004.

[20] WengGuirong, Su Jian, "Microarray Images Processing Using the Offset Vector Field and Expectation Maximization Algorithm,"*IEEE conference*, 2010.

[21] Manjunath S S and LalithaRangarajan, "Refinement of K-means Clustering for Segmentation of Microarray Images,"*Journal of Convergence and Information Technology*, vol. 6(9), pp. 403-411, 2011.

[22] J. Harikiran, *et al.*, "Fuzzy C-means with bidimensionaempirical mode decomposition for segmentation of microarray image," *International Journal of Computer Science*, vol. 9, pp. 189–198, Sep 2012.

[23] N Karimi, S Samavi, S. Shirani, "Segmentation ofDNA microarray Images using adaptivegraph based method,"*IET Image processing*, vol. 4, pp. 19-27, 2008.

[24] ShenghuaN, Pan Wang, "Spotted Cdna Microarray Image Segmentation Using ACWE,"*RomanianJournal Of Information Science And Technology*, vol. 12(2), pp. 249-263, 2009.

[25] Yan Zhang, Bogdan J. Matuszewski, Lik-Kwan Shark, "Medical Image Segmentation Using New Hybrid Level-Set Method,"*Fifth International Conference BioMedical Visualization: Information Visualization in Medical and Biomedical Informatics*, vol. 2, pp. 89-97, 2009.

[26] Kaustubha A. Mendhurwar, RajasekharKakumani,VijayDevabhaktuni, "Microarray image segmentation using chan-vese active contour model and level set method," *Annual International Conference of the IEEE Engineering in Medicine and Biology Society*, vol. 5, pp. 256-264, 2009.

[27] Eleni Zacharia and Dimitris Maroulis, "3-D Spot Modeling for Automatic Segmentation ofcDNA Microarray Images," *IEEE Transactions onNanobioscience*, vol. 9(3), 2010.

[28] Guifang Shao, Tingna Wang, Wupeng Hong, Zhigang Chen, "An Improved SVM Method for eDNAMicroarrayImage Segmentation," *The 8th International Conference on Computer Science & Education*, 2013.

[29] Chunming Li, *et al.*, "A Level Set Method for Image Segmentation in the Presence of Intensity Inhomogeneitieswith Application to MRI,"*IEEE Transactions on Image Processing*, vol. 20, pp. 2007-2017, 2011.

[30] StamosKatsigiannis, Eleni Zacharia, and Dimitris Maroulis, "Grow-Cut Based Automatic cDNA Microarray Image Segmentation,"*IEEE Transactions On Nanobioscience*, vol. 14(1), 2015.

[31] Guifang Shao, Shunxiang Wu, Tiejun Li, "cDNA Mieroarray Image Segmentation with an Improved Moving K-means Clustering Method,"*IEEE ICSC*, 2015.

[32] MachmudR Allahamdi, Ito Wasito, "Improved Micro array images Cancer Classificationusing k-nearest neighbour using particle swarm optimization," *IEEE conference*, *IWBIS*, 2017.

[33] T. Barrett, S. E.Wilhite, P. Ledoux, *et al.*, "NCBI GEO: archive for functional genomics data sets—update," *Nucleic Acids Research*, vol. 41, pp. 991–995, 2013.

[34] Pascal Getreuer, "Chan-Vese Segmentation," *IPOL Journal Image Processing On Line*, vol. 2, pp. 109-129, 2014.

[35] Mohammed M. Abdelsamea ,Giorgio Gnecco, Mohamed Medhat Gaber, "A SOM-based Chan–Vese model for unsupervised image segmentation," *Springer-Verlag Berlin Heidelberg 2015*, *Soft Comput (2017)*,no. 21, pp. 2047–2067, 2015.

[36] Byeongkeun Kang, Truong Q N, "Random Forest with Learned Representations for Semantic Segmentation*,"IEEE Transactions on Image Processing: a Publication of the IEEE Signal Processing Society*, pp. 1-1, 2019.

[37] Z. Wang, B. Zineddin, J. Liang, *et al.*, "A novel neural network approach to cDNAmicroarray image segmentation," *Computer Methods and Programs in Biomedicine*, vol. 11, pp. 189–198, Jul 2013.

[38] N. Giannakeasa, *et al.*, "Segmentation of microarray images using pixel classification-comparison withclustering-based methods," *Computers in Biology and Medicine*, vol. 43(6), pp. 705–716, 2013.

[39] Karthik S A and Manjunath S S, "An Enhanced Approach for Spot Segmentation of MicroarrayImages," *International Conference on Computational Intelligence and Data Science Procedia Computer Science*,vol. 132,pp.226–235, 2018.
[40] Yongsheng Pan, Yong Xia, Dinggang Shen, "Foreground Fisher Vector: Encoding Class-Relevant Foreground to Improve Image Classification," *IEEE Transactions on Image Processing: a Publication of the IEEE Signal Processing Society,* pp. 1-1, 2019.
[41] Tingman Yan, Yangzhou Gan, Zeyang Xia, Qunfei Zhao, "Segment-based Disparity Refinement with Occlusion Handling for Stereo Matching," *IEEE Transactions on Image Processing: a Publication of the IEEE Signal Processing Society*,pp. 1-1, 2019.
[42] Wei Wang, *et al.*, "Structural Similarity based Nonlocal Variational Models for Image Restoration," *IEEE Transactions on Image Processing: a Publication of the IEEE Signal Processing Society*, pp. 1-1, 2019.

BIOGRAPHIES OF AUTHORS

Karthik S A has received B.E (ISE) degree in 2011 and M.Tech (CNE) degree in 2013 from Visvesvaraya Technological University Belgaum, Karnataka, India. Currently he is working as Assitant Professor in Dept. of ISE at Dayananda Sagar Academy of Technology and Management, Bangalore, Karnataka, India and pursuing PhD in Visvesvaraya Technological University. His areas of interest include microarray image processing, Datamining and Machine learning.

Dr. Manjunath S. S B.E (CSE), M. Tech (Networking), Ph.D(CSE). He is working as Professor & HOD in Dept. of Computer Science and Engineering ATME, Mysore, Karnataka. He has 17+ years experience in teaching, R&D and Administration. He has published more than 41 papers in International/National Journals and conferences. His areas of interest are image processing and networking.