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Comparative Study of IAFCM & SSFCM Segmentation Techniques for Analysis of M-FISH Chromosome Images

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Abstract: ***Analysis of Chromosomes is an important and difficult task for clinical diagnosis and biological research. Conventional analysis of chromosomes using gray scale images is a complex and tough task. With the arrival of multi-spectral image acquisition since 1996, chromosome analysis becomes much easier using M-FISH (Multi-spectral Fluorescence In-Situ Hybridization) chromosome images. In this paper IAFCM(Improved Adaptive Fuzzy C mean Clustering) & SSFCM(Spatial & Spectral Fuzzy C Mean Clustering) algorithms are used for segmentation. Some noise removal techniques like Pre-processing are also applied to improve segmentation accuracy of M-FISH chromosome images.***
Index Terms**: Chromosomes, M-FISH, FCM, spatial information.***

I. INTRODUCTION

Chromosomes are threadlike structures of nucleic acids & protein found in nucleus of most living cells, carrying genetic information in the form of genes . Normally human has 46 chromosomes which are arranged into 23 pairs. The first 22 pairs are called autosomes, 23rd pair is sex chromosome, which is either XX or XY. Chromosome analysis is a study about the number and general structure of chromosomes. The main goal of chromosome analysis is karyotyping, which is the arrangement of chromosomes in decreasing order of their size in pairs. In a standard karyotype, chromosomes from the cells in the body are counted to ensure that evaluated cells have a correct number of chromosomes, and their structure is evaluated to guarantee that there are no missing, extra (duplicated), or rearranged part of materials in chromosomes. Since chromosome abnormalities cause many genetic diseases such as mental retardation, autism, and inborn errors of metabolism, chromosome analysis is performed mainly for disease diagnosis. In 1996, a new technique called M-FISH was developed, which is a combinatorial labeling technique for simultaneous analysis of chromosomes. This technique makes use of dyes (fluorophores) to label chromosomes. Dyes are stained with a particular class of chromosomes, and hence each class of chromosome has a unique combination of fluorophores. To label, all the 24 classes of human chromosomes (22 homologous pairs, X chromosome, and Y chromosome) at least five fluorophores are required. With p fluorophores we can label 2^p objects. One fluorophore DAPI (4', 6- Diamidino-2-phenylindole) attaches with all chromosome parts is used to generate a gray scale image of chromosomes.[3] Five additional fluorophores are used to distinguish different classes of chromosomes. In this paper, M-FISH image segmentation is obtained by applying IAFCM & SSFCM Algorithms.Some pre-processing techniques are applied to improve the accuracy of segmentation. In Section 2 we include the literature survey conducted for the Pre-processing and segmentation of M-FISH chromosome images. The methodology used for Pre-processing & segmentation method is explained in Section 3 followed by results of experiments in Section4. We conclude the paper in Section 5.

II. LITERATURE SURVEY

Lijiya A., Sangeetha M.K., and V.K.Govindan, [1] presented a few important works in chromosome labeling. Use of unsupervised classification method for fuzzy logic classification is presented by H.Choi in [2]. This paper also presents a new segmentation method that combines both spectral and edge information[2].An automated registration technique to correct misalignment across the different fluor images caused by chromatic aberration and other factors are discussed in[3].[4] In the paper, author have introduced two novel approaches for multichannel image registration and feature selection. Color compensation techniques are explained in [5].In this author conclude that by analyzing the intensity combinations of each pixel, all chromosome pixels in an image are classified. Use of Discrete Wavelet Transform (DWT) and Bayes rule for M-FISH image segmentation and classification is presented in [6].Classification of multicolor fluorescence in situ hybridization images using Gaussian mixture models is discussed in [7]. Use of support vector machines (SVM) for segmentation and classification of M-FISH image was described in [8]. They also compared result of SVM with Bayesian classifier. Image normalization issues such as background subtraction, multispectral

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channel image registration and dimension reduction, which can lead to improved accuracy of pixel classification is discussed in [9]. Later, Cao and Wang [10] presented Segmentation of M-FISH images for improved classification of chromosomes with an adaptive Fuzzy C-Means clustering. Adaptive FCM was done by incorporating a gain field which models and corrects intensity homogeneity and also regulates center of each intensity cluster. Intensity homogeneity is mainly caused by the image acquirement and uneven hybridization. It provides lowest segmentation and classification error and is better than FCM and AFCM. Overlapping and touching chromosomes are still a problem in pixel-by-pixel classification. Many researchers have attempted to sort out this issue. Some of the important work in this category is [11, 12, 13]. In [11], minimum entropy is used as the main segmentation criterion to overcome overlapping and touching chromosome images. However, the computational time and complexity is very high; performance is very sensitive to its parameters and the approach is tested only on small number of images. From the above review work on the various approaches suggested by the researchers, one can conclude that region-based classification approaches are superior to pixel by pixel approach in terms of accuracy and computational time.[14] In this the chromosomal image is segmented while integrating both spatial & spectral information.[15]The publicly available database which contains a set of chromosomal images which can be used to check & compare the results of different algorithms.

III. METHODOLOGY

A. Pre-Processing

Pre-processing is applied to image at the lowest level of abstraction. The aim of Pre-processing is an improvement of the image data that reduces unwanted distortions or enhances some image features important for further processing. Here, The Median filter is used to achieve Pre-processing. The Median filter is a non-linear digital filtering technique often used to remove noise from an image. Such noise reduction is a typical Pre-processing step to improve the results of later processing. Fig.1. Shows input chromosome image and that input image is pre-processed using the median filter. The output of pre-processing is shown in Fig.2.

B. Segmentation

Image segmentation is the process of dividing a digital image into multiple segments. The aim of segmentation is to clarify and change the representation of an image into something that is more significant and easier to analyze. Image segmentation is normally used to discover objects and boundaries of image. More precisely, Image segmentation is the process of allocating a label to every pixel in an image such that pixels with the same label share certain attribute. Here, two different algorithms are used for segmentation.

To overcome the noise acuteness of conventional Adaptive fuzzy c-means (AFCM) clustering IAFCM algorithm & SSFCM algorithm for image segmentation are presented in this paper.

1) Improved adaptive fuzzy c-means algorithm.

The objective function of the IAFCM algorithm is introduced as follows:

$$A_{IAFCM} = \sum_{k\epsilon D}\sum_{p=1}^{NC} U_{kp}^{q} ||Yk - Gk\ Cp||^2 + \lambda \sum_{k\epsilon D}(Gk - (L * G)k)^2 \quad (1)$$

Where U_{kp} is the membership function with positive values between 0 and 1; Yk is the observed image intensity at location k; Cp is the cluster centers; q is a weighting proponent on each fuzzy membership, which decide the amount of fuzziness; *D* is the whole area of image; NC is the number of clusters.[10]

{Gk |k∈ D} is the gain field to be found, and L is a (2r +1)*(2r + 1) average convolution kernel given by

$$L = \frac{1}{N}\begin{bmatrix} 1 & \cdots & 1 \\ \vdots & 1 & \vdots \\ 1 & \cdots & 1 \end{bmatrix}$$

where $N = (2r + 1)*(2r + 1)$ is the number of pixels within area D_{kr}. Let us write the regulation term in (1) as A_M ,

$$A_M = \sum_{k\epsilon D}(Gk - (L * G)k)^2 \quad (2)$$

and define the modified FCM objective function A_X as

$$A_X = \sum_{k\epsilon D}\sum_{p=1}^{NC} U_{kp}^{q} ||Yk - Gk\ Cp||^2 \quad (3)$$

Thus, (1) could be rewritten as follows,

$$A_{IAFCM} = A_X + \lambda A_M \quad (4)$$

The objective function that is given by (4) is taken as a conditional minimization problem with the constraint function, which can be formulated as Lagrange multipliers form as A_M. To solve this conditional minimization problem, a necessary condition is that the gradient is zero.

$$\partial A_{IAFCM} / \partial U_{kp} = 0;\ k \in D$$

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$\partial A_{IAFCM} / \partial U_{Cp} = 0;\ k \in D$
$\partial A_{IAFCM} / \partial UG_k = 0;\ k \in D$
$\partial A_{IAFCM} / \partial \lambda = 0;\ k \in D$
By solving this we get the equations for U_{kp},C_p & G_k.

a) Step 1. Compute G_k with k(k =1,2,3.....n) & cluster centers C_p with p(p = 1,2.....NC) where NC is the no. of clusters.
b) Step 2. Modernize the membership function $U_{kp.}$
c) Step 3. Modernize the cluster centers $C_{p.}$
d) Step 4. Compute & update the gain field $G_{k.}$
e) Step 5. If the maximum change of U_{kp} is less than tolerance & the maximum change of G_k is less than tolerance then break. Otherwise, go to step 2. The output of Segmentation using IAFCM algorithm is shown in Fig.3.[10]

2) *Spatial & Spectral adaptive fuzzy c-means Algorithm:* In SSFCM, the chromosomal image is segmented by integrating both spatial and spectral information. The term spatial refers to space. The spatial domain is the normal image space, where some magnitude is allotted to different positions(x,y).Spatial information improves the quality of clustering which is not utilized in the conventional FCM & Improved adaptive FCM. Normally fuzzy c-means (FCM) algorithm is not powerful against noise. This paper presents a modified version of improved fuzzy c-means (FCM) algorithm that integrates both spatial information and spectral information into the membership function for clustering of images. The spatial function is the aggregate of the membership function in the neighborhood of each pixel under application. The ADMM (Alternating direction method of multipliers) algorithm is used for optimization of the image. The ADMM is an algorithm that solves cambered optimization problems by breaking them into smaller pieces. These pieces are easier to handle. The advantages of this new method are:

a) It produces regions more correlative than those of other methods for images.
b) It reduces the false blobs; and
c) It eliminates noisy spots. It is less conscious to noise as compared with other techniques.

The objective function of the Spatial and spectral fuzzy C means introduced as follows:

$$\sum_{p=1}^{MD}\sum_{q=1}^{NC}\sum_{r=1}^{N}\mu_{pqr}\left\|x_{pr}-c_{pq}\right\|^2+\lambda\|U\mathrm{D}\|_{2,1}$$

Subject to $\sum_{q=1}^{NC}\mu_{pqr}=1$

Where, MD denotes dimension of data, NC denotes no. of clusters, N denotes no. of pixels in the image, μ_{pqr} denotes the value of membership function corresponding to the p-th channel, the q-th cluster, and the r-th pixel. U denotes the matrix of the membership function. The output of Segmentation using SSFCM algorithm is shown in Fig.4.[14]

IV. RESULTS AND ANALYSIS

A. *Figures*

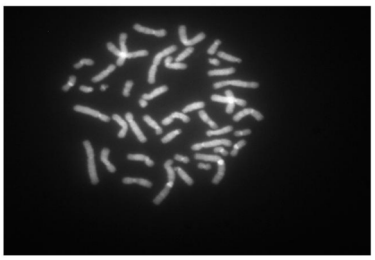


Fig.1. Input Image

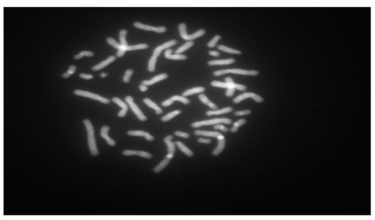


Fig.2.Preprocessed Output

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Fig.3.IAFCM Output

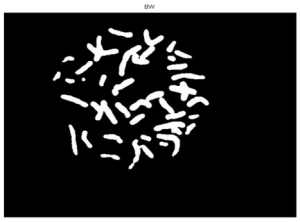


Fig.4.SSFCM Output

Image	PR		IR	
	IAFCM	SSFCM	IAFCM	SSFCM
1	0.656988245	0.865997577	0.522097	0.134002
2	0.98706446	0.984056232	0.012936	0.016202
3	0.982047842	0.986174484	0.017952	0.014019
4	0.956836141	0.981057559	0.043164	0.019308
5	0.805526545	0.989628442	0.241424	0.01048
6	0.988812392	0.986543278	0.011188	0.01364
7	0.990572221	0.990264603	0.009428	0.009831
8	0.954698582	0.973588814	0.045301	0.027128
9	0.975732506	0.988415673	0.024267	0.01172
10	0.988912222	0.992745163	0.011088	0.007308

B. Analysis

To check the performance of both segmentation methods named as IAFCM & SSFCM, Perfect ratio(PR) and Imperfect ratio(IR) are analyzed. PR and FR are calculated by using the following equations:

Perfect ratio (PR) = $\frac{Chromosome\ pixels\ perfectly\ segmented}{Total\ chromosome\ pixels}$

Imperfect ratio (IR) = $\frac{chromosome\ pixels\ imperfectly\ segmented}{Total\ chromosome\ pixels}$

The above IR equation chromosome pixels imperfectly segmented are nothing but background pixels that are segmented as a chromosome. From PR and IR equations, it can be noticed that a correct segmentation should give greater PR and smaller IR.[10]

V. CONCLUSION

In the classification of a chromosomal image, image segmentation is one of the important steps. To raise the accuracy of classification, image segmentation has to be improved. The proposed methodology concludes that the SSFCM algorithm has the advantage over IAFCM as we get a higher percentage of PR in SSFCM than IAFCM. To improve segmentation accuracy an optimization technique is used. To achieve optimization ADMM algorithm is used which proceeds diagonally hence numbers of iterations are reduced. Therefore if a chromosomal image is segmented by incorporating both spatial and spectral information, then it improves classification accuracy, and it becomes easy for doctors to analyze an image.

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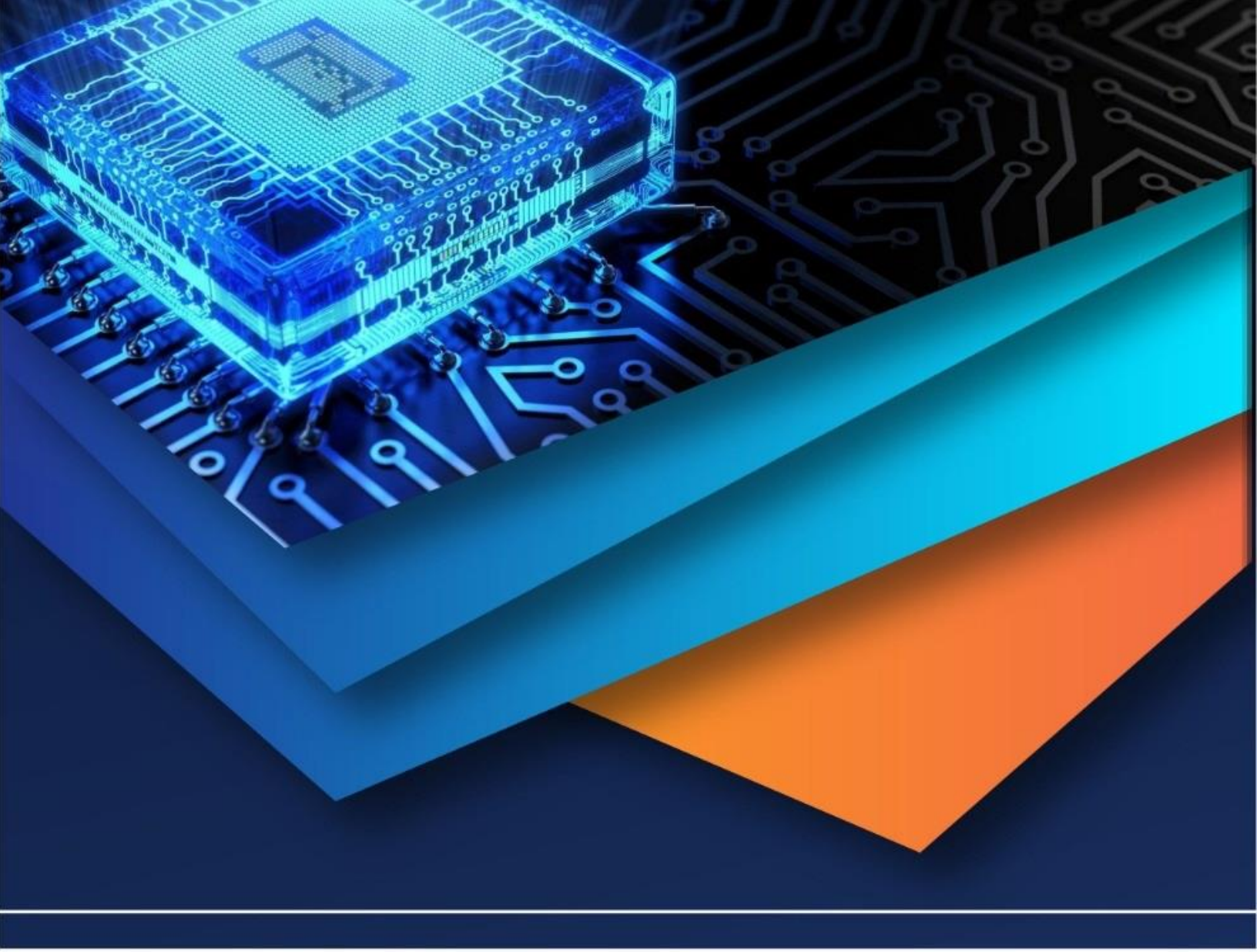
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