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Segmentation Approaches for Human Metaspread Chromosome Images Using Level Set Methods

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Abstract. The human metaspread images contain the chromosomes of an individual that are imaged during the metaphase stage of cell division. The chromosomes are the genetic information carriers, any alteration either in the number of chromosomes or the structure of chromosomes, results in a medical condition termed as genetic defects. The genetic defects can be cause of many diseases that are difficult to cure. In order to uncover the genetic defects the metaspread images are segmented, to count the number of chromosomes present or to study the structure of the chromosomes. The metaspread images suffer from intensity in homogeneity, because of which it is very difficult to extract the individual chromosomes by using conventional segmentation techniques. In this work various segmentation approaches are implemented and studied, to segment the chromosomes from the metaspread images. The minimization of region scalable fitting energy for image segmentation proposed by Chumming li et. al, is effective in segmenting the metaspread images that have intensity in homogeneity, as this technique uses the local intensity values of the nearby regions of the objects and find the approximate intensity values along both sides of the contour. The paper compares the level set based segmentation algorithms based upon their implementation techniques and the segmentation results. The methods have been compared in terms of number of objects segmented, time taken, complexity, type of segmentation approach. The ADIR dataset of metspread images has been taken for the purpose of experimentation.

Keywords: Metaspreads, Chromosomes, Segmentation, Level Set Methods.

1   Introduction

The human metaspread images contain the chromosomes that are imaged using a light microscope[29] during metaphase of mitosis. The cells are usually imaged during the metaphase or the pro metaphase as during this phase only the chromosomes are visible as distinct bodies and can give useful information[18].The cells that are imaged for the metaspread image generation are generally taken from the samples of blood, product of conception, amniotic fluid, tissues or bone marrow[6][19][32]. A normal healthy human being has 23 pairs of chromosomes out of which 22 pairs are homologous and the 23rd pair has sex determining chromosomes that are either XX or XY for females and males respectively[20].Any alteration either in structure or the number of the chromosomes is termed as genetic defect[14]. The genetic defects are the underlying cause of mental retardation, frequent miscarriage, epileptic seizures or various forms of cancers[15].

In order to extract the chromosomes from the metaspread images, the human metaspread images undergo the segmentation process. The human metaspread images suffer from intensity in homogeneity[16] due to which the conventional segmentation approach based upon global thresholding[31] are not suitable for metaspread image segmentation, as in case of very small chromosomes or towards the tips of the chromosomes the intensity is very low. The global thresholding based methods can miss these portions. Secondly the chromosomes are non-rigid objects and are generally present in various orientations[33], they may be bent, touching each other or may be overlapping[5], global thresholding based method are not able to segment these types of chromosomes and thus can segment them as a cluster of chromosomes as evident from the fig 1 below.
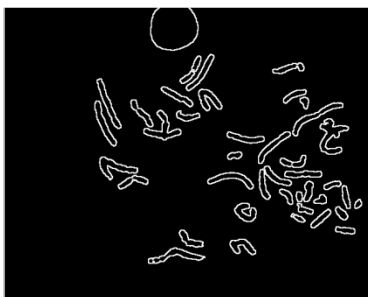

Fig. 1 Metaspread Image as segmented by Otsu method

From last so many years many methods have been proposed for the segmentation of chromosome metaspread images. Most of the methods have used Otsu thresholding based methods for the purpose of segmentation[4][41][21][43].

The Otsu segmentation approach is based upon the global thresholding approach of segmentation, this technique is not well suited for segmentation of metaspread images, as metaspread images have intensity in homogeneity and thus results in either over segmentation or under segmentation.

In order to deal with the drawbacks of global thresholding, a local thresholding based approach termed as adaptive thresholding was proposed[44]. This technique is

quite successful in segmenting the images that have intensity inhomogene-ity[34][9][28][17] but it fails in case of chromosomes that are lying close to each other, it segments them as a cluster of chromosomes. In a way it under segments the metaspread images for the objects that are lying close to each other. The segmentation results for metaspread images for adaptive thresholding are illustrated in fig 2 below.
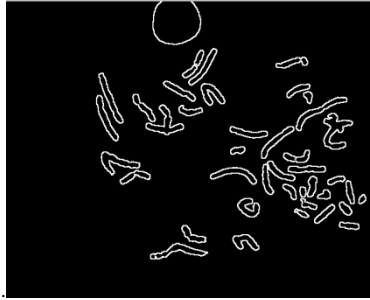

Fig. 2 Segmentation of metaspread images using adaptive thresholding

Watershed transform based segmentation techniques have also been used for seg-menting the metaspread images[22][23][40][3][2] these techniques find the distance transform of the image to determine the number of regions to be segmented, this results in over segmentation due to local minima's, so in order to avoid local minima's before applying the watershed segmentation the negative distance transformed image is recon-structed. The watershed based segmentation technique is computationally expensive; it results in under segmentation of the metaspread images.
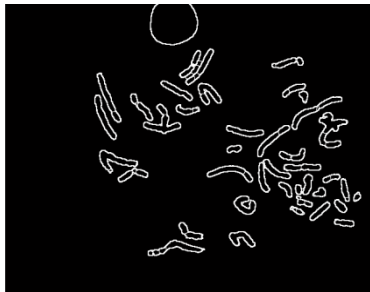

Fig. 3 Results of segmentation after application of watershed method

Most of the previously reported methods have either used Otsu method, adaptive thresholding or watershed methods for the segmentation of the chromosomes, and much effort has been done in reconstructing the overlapping and touching chromosomes as reported in[38][37][39][11][35]. Since the metaspread images as shown in fig 3 below, contains intensity in homogeneities, the level set based segmentation techniques have proved to be quite fruitful to segment the metaspread chromosome images as reported in[34]. These techniques segment the images by drawing a smooth contour around the boundaries of the object and are very efficient in identifying the objects present in the image irrespective of the shape and size. Over the years many level set based methods

have been proposed for image segmentation. In this work an effort has been made to compare and contrast various level set based segmentation algorithms, and propose the most suitable algorithm for the segmentation of metaspread images.
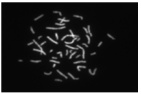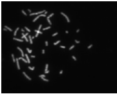
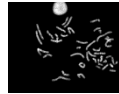| Image						
Image name	A0102XYD	A020315D	P0802XYD	P110121D	P110536D	P1102XYD

Fig. 4 Sample metaspread images depicting intensity inhomogeneity

The rest of the paper is organized as follows: section 2 explains material and methods, section 3 has results and discussion and section 4 concludes the paper.

2   Material and Methods

2.1   Dataset Used

In order to evaluate the various segmentation algorithms the DAPI images of the ADIR dataset[46]has been used. The dataset has 200 images. For the purpose of our study we have taken all the images. The result that are presented in this paper are for a single image named as P110536D as shown in fig 3 above.

2.2   Performance Evaluation

The performance of segmentation can be evaluated by comparing the segmentation results with the ground truth information. The measure for performance evaluation is accuracy, which can be calculated as follows:

$$Accuracy = \frac{Count\ of\ correctly\ segmented\ individual\ \ objects}{Total\ Number\ of\ individual\ Objects} \tag{1}$$

The results of the segmentation have been compared with the ground truth information provided by experienced cytogeneticists.

2.3   Methods

This section describes the various segmentation techniques that have been taken for the purpose of segmentation of the metaspread images. The proposed techniques have been implemented using the MATLAB 2014 as per their original manuscript details.

The software named as Creatis [7] has been used for the evaluation of some of the methods.

2.3.1 Level Set Based Segmentation

Level set methods are a kind of deformable models for segmentation of images, in which the initial level set is initialized, then based upon the properties of the image and the level set, the level set is recomputed, and is moved towards the boundaries of the objects of the image[13][42]. There are generally two type of properties that helps in the evolution of the level set, one is based upon the internal properties defined within the level set that helps the boundaries of the curve to be smooth and the second are the external properties that are dependent upon the image, that helps the curve to be more towards the boundaries of the objects in the image[42]. They are based upon implicit deformable models and are capable of handling the topological variations[3]. Over the years many level set based algorithms have evolved for the segmentation of images that suffer from intensity inhomogeneity[25]. In this paper 10 level set based methods have been compared.

2.3.1.1 Geodesic Active Contours[10]

This method is based upon image gradient, which is used to calculate the force function. The curve is converged towards regions of higher gradient value. It has a inbuilt regularization term, the curve evolution equation is calculated using distance function, every iteration re initialise the evolution equation.

The energy for the level set is calculated using the following equation

$$E_t(\Gamma) = \int_0^1 g_t\left(Im(\Gamma(q))\right)\|\Gamma'(q)\|dq \tag{2}$$

Where

$$g_t(Im) = \frac{1}{1+\|\nabla(G*Im)\|^2} \tag{3}$$

$Im(.)$ Is the intensity of the image I, Γ represents the parametric curve, and G is the filter of the type Gaussian with unit variance.

The evolution equation for this method is

$$\frac{\partial\phi}{\partial t}(x) = g_t\left(Im(x)\right)\|\nabla\phi(x)\|(c+\kappa) + \nabla g_t\left(Im(x)\right)\nabla\phi(x) \tag{4}$$

Where $\kappa = div\left(\frac{\nabla\phi(x)}{\|\nabla\phi(x)\|}\right)$ represents the curvature of the emerging curve and c is the balloon force and a constant value that is set to 1.

2.3.1.2 Active Contours without Edges[12]

This approach aims at dividing the image into two regions that are homogeneous based upon the average value. This method is sensitive to initialization as the evolution equation is computed on level sets, one of the narrow bands. The curve evolution equation is re computed for each iteration.

The energy for the level set is calculated using the following equation:

$$E_t(\phi) = \int_\Omega F_t\big(Im(x), \phi(x)\big)\, dx + \lambda \int_\Omega \delta\big(\phi(x)\big)\, \|\nabla\phi(x)\|\, dx \quad (5)$$

Where δ represents the dirac function and

$$F_t\big(Im(x), \phi(x)\big)\, dx = H_t\big(\phi(x)\big)(Im(x) - v)^2$$
$$+ \Big(1 - H_t\big(\phi(x)\big)\Big)\big(Im(x - u)\big)^2 \quad (6)$$

Where H is Heaviside function, $v\ and\ u$ are the parameters to be updated on every iteration

$$u = \frac{\int_\Omega \Big(1 - H_t\big(\phi(x)\big)\Big).Im(x)\, dx}{\int_\Omega 1 - H_t\big(\phi(x)\big)\, dx} \quad (7)$$

$$v \frac{\int_\Omega \Big(H_t\big(\phi(x)\big)\Big).Im(x)\, dx}{\int_\Omega H_t\big(\phi(x)\big)\, dx} \quad (8)$$

$E(\phi)$ is a combination of data attachment term and regularization term that aims at minimizing the length of the contour and smoothing it during its evolution. The evolution equation for this method is as follows:

$$\frac{d\phi}{dt} = \delta\big(\phi(x)\big)\big((Im(x) - v)^2 - (Im(x) - u)^2\big) + \lambda\delta\big(\phi(x)\big)\kappa \quad (9)$$

2.3.1.3 Minimization of Region Scalable Fitting Energy for Image Segmentation[26]

The proposed technique overcomes the segmentation difficulties that arise due to intensity inhomogeneity. It is based upon the region based active contours, in which the local intensity information is retrieved at a controllable scale. The energy criterion is defined in form of a contour and in addition to that two local energy fitting functions are used to find the local intensities along both sides of the contour. A level set formulation is created with level set regularization term using which the equation of the curve is evolved for minimization of the energy. The extraction of the local fitting energy helps in dealing with the intensity in homogeneity issue. There is no re-initialization of the level set function as it is preserved by the regularization term. The evolution equation is based upon the signed distance transform.

The energy criterion is based upon the following equation:

$$E_t(\phi) = \lambda_1 \int_\Omega \int_\Omega K_{t_\sigma}(x$$
$$- y)|Im(y) - f_1(x)|^2 H_t(\phi(x)) \, dy \, dx + \lambda_2 \int_\Omega \int_\Omega K_{t_\sigma}(x$$
$$- y)|Im(y) - f_2(x)|^2 \left(1 - H_t(\phi(x))\right) dy \, dx$$
$$+ v \int_\Omega \delta(\phi(x))\|\nabla\phi(x)\| \, dx$$
$$+ \mu \int_\Omega \frac{1}{2} (\|\nabla\phi(x)\| - 1)^2 \, dx \tag{10}$$

Where $Im(x)$ is the intensity of the image, H is the Heaviside function, K_σ is the Gaussian kernel.

$$K_{t_\sigma}(u) = \frac{1}{(2\pi)^{n/2} \sigma^n} e^{-\|u\|^2/2\sigma^2} \tag{11}$$

Where σ is a scale parameter greater than 0 and f_1, f_2 are the functions for the pixel located at x and calculated for each iteration as:

$$f_1 = \frac{K_{t_\sigma} * \left(H_t(\phi(x))Im(x)\right)}{K_{t_\sigma} * H_t(\phi(x))} \tag{12}$$

$$f_2 = \frac{K_{t_\sigma} * \left(1 - H_t(\phi(x))Im(x)\right)}{K_{t_\sigma} * \left(1 - H_t(\phi(x))\right)} \tag{13}$$

λ_1 and λ_2 are the constants. The energy term has four integrals, the first and second represents the data terms; the third is regularization term used to smoothen the curve and fourth is the regularization term that forces the level set to maintain the signed distance properties during the evolution.

The evolution equation used for this method is :

$$\frac{\partial\phi}{\partial t} = \delta(\phi(x)) \left(\lambda_1 \int_\Omega K_{t_\sigma}(x - y)|Im(y) - f_1(x)|^2 \, dy\right.$$
$$\left. + \lambda_2 \int_\Omega K_{t_\sigma}(x - y)|Im(y) - f_2(x)|^2 \, dy\right)$$
$$+ v\delta(\phi(x))\kappa + \mu(\nabla^2\phi(x) - \kappa) \tag{14}$$

2.3.1.4 Localizing Region Based Active Contours[24]

It is a region based segmentation approach, in which local image information is considered and the contour is evolved using the local information. The heterogeneous image can be segmented due to localized contours. This method is sensitive to initialization. The evolution function is based upon distance transform and is recomputed for every iteration.

The energy criteria for this method are:

$$E_t(\phi) = \int_\Omega \delta\big(\phi(x)\big) \int_\Omega B_t(x,y) \cdot F_t\big(Im(y),\phi(y)\big) \, dy \, dx$$

$$+ \lambda \int_\Omega \delta\big(\phi(x)\big) \|\nabla\phi(x)\| \, dx \qquad (15)$$

Where Dirac function is denoted by δ and B represents the Ball function with radius r for the point x, it is defined as:

$$B_t(x,y) = \begin{cases} 1, \|x - y\| \leq r \\ 0, otherwise \end{cases} \qquad (16)$$

and

$$F_t\big(Im(y),\phi(y)\big)$$
$$= \begin{cases} \Big(H_t\big(\phi(y)\big)\big(Im(y) - v(x)\big)^2 + \big(1 - H_t\big(\phi(y)\big)\big)\big(Im(y) - u(x)\big)^2, \\ \qquad\qquad ChanVese features \\ \big(v(x) - u(x)\big)^2 \ Yezzi features \end{cases} \qquad (17)$$

Where H represents the Heaviside function, $v(x)$ and $u(x)$ are the functions that are to be updated at each iteration and are defined as follows:

$$u(x) = \frac{\int_\Omega B_t(x,y) \cdot \big(1 - H_t\big(\phi(y)\big)\big) \cdot Im(y) \, dy}{\int_\Omega B_t(x,y) \cdot \big(1 - H_t\big(\phi(y)\big)\big) \, dy} \qquad (18)$$

$$v(x) = \frac{\int_\Omega B_t(x,y) \cdot H_t\big(\phi(y)\big) \cdot Im(y) \, dy}{\int_\Omega B_t(x,y) \cdot H_t\big(\phi(y)\big) \, dy} \qquad (19)$$

The energy term is the combination of data term and the regularization term. The evolution equation for the method is given by the equation as below:

$$\frac{\partial\phi}{\partial t}(x) = \delta\big(\phi(x)\big) \int_\Omega B_t(x,y) \cdot \nabla_\phi F_t\big(I(y),\phi(y)\big) \, dy + \lambda\big(\delta(x)\big)\kappa \ (20)$$

Where

$$\nabla_\phi F_t\big(I(y), \phi(y)\big)$$

$$= \begin{cases} \delta\big(\phi(y)\big) \Big(\big(I(y) - v(x)\big)^2 - \big(I(y) - u(x)\big)^2\Big) \ \text{Chan } Vese feature, \\ \delta\big(\phi(y)\big) \left(\dfrac{\big(I(y) - v(x)\big)^2}{A_v} - \dfrac{\big(I(y) - u(x)\big)^2}{A_u}\right) \text{Yezzi feature,} \end{cases} \quad (21)$$

A_v and A_u represent the local exterior and interior areas as calculated by:

$$A_u = \int_\Omega B_t(x, y) \cdot \Big(1 - H_t\big(\phi(x)\big)\Big) dy \qquad (22)$$

$$A_v = \int_\Omega B_t(x, y) \cdot \Big(H_t\big(\phi(x)\big)\Big) dy \qquad (23)$$

2.3.1.5 A Real Time Algorithm for the Approximation of Level Set Based Curve Evolution[36]

This is a very fast approximation algorithm based upon curve evolution for level sets. It is a two-step method and is independent of partial differential equations. First step aims at evolution of the curve for the data dependent parameter and the second step smoothens the regularization term. The evolution equation for the proposed method can be represented as below:

$$F_t\big(Im(x), \phi(x)\big)$$
$$= H_t\big(\phi(x)\big)(Im(x) - v)^2$$
$$+ \Big(1 - H_t\big(\phi(x)\big)\Big)(Im(x) - u)^2 \qquad (24)$$

Where H is Heaviside function, u and v represents the parameters that are recomputed for each iteration as per equations of Chan Vese model.

2.3.1.6 Variational B-Spline Level Set[8]

This is a region based segmentation method that aims at dividing the input image into two classes of homogeneous regions. In this the energy computation function is a continuous parametric based upon B-Splines. The minimization term is computed with the coefficients of the B Spline.

The energy criterion for this model is given by the equation below:

$$E_t(\phi) = \int_\Omega F_t\big(Im(x), \phi(x)\big) dx, \qquad (25)$$

The data attachment term is given by

$$F_t\big(Im(x), \phi(x)\big)$$
$$= H_t\big(\phi(x)\big)(Im(x) - v)^2$$
$$+ \Big(1 - H_t\big(\phi(x)\big)\Big)(Im(x) - u)^2 \tag{26}$$

Where H is Heaviside function, u and v represents the parameters that are recomputed for` each iteration as per equations of Chan Vese model.

The minimization of the energy function can be carried with respect to the coefficients of B-spline c[k]. The derivatives can be expressed using the equation given as under:

$$\frac{\partial E}{\partial c\big[K_{t_o}\big]} = \int_\Omega \frac{\partial F_t\big(x, \phi(x)\big)}{\partial \phi(x)} \cdot \beta^n \Big(\frac{x}{h} - K_{t_0}\Big) dx \tag{27}$$

With

$$\frac{\partial F_t\big(x, \phi(x)\big)}{\partial \phi(x)} = \delta\big(\phi(x)\big)\big((Im(x) - v)^2 - (Im(x) - u)^2\big) \tag{28}$$

The level set evolution can be calculated using the gradient descent on the coefficients of B-spline. The coefficients of B-spline are given as:

$$c^{i+1} = c^i - \lambda \nabla_c E_t\big(c^i\big) \tag{29}$$

λ represents the step of the iteration and ∇_c denotes the gradient energy.

2.3.1.7 Active Contours with Selective Local or Global Segmentation: A New Formulation and Level Set Method[45]

It is a region based active contour technique for image segmentation. It uses a binary level set function and a Gaussian based regularization term. It uses a region based function which stops the contours at weak edges. It can perform local as well as global segmentation. The signed pressure force (SPF) function is calculated using the equation below:

$$spf_t\big(Im(x)\big) = \frac{Im(x) - \dfrac{c_1 + c_2}{2}}{\max\left(\left|Im(x) - \dfrac{c_1 + c_2}{2}\right|\right)} \quad x\epsilon\Omega, \tag{30}$$

Where c_1 and c_2 are calculated as

$$c_1(\phi) = \frac{\int_\Omega Im(x) \cdot H_t(\phi)\, dx}{\int_\Omega H_t(\phi)\, dx} \tag{31}$$

$$c_2(\phi) = \frac{\int_\Omega Im(x) \cdot (1 - H_t(\phi)\, dx)}{(1 - H_t(\phi)\, dx)} \tag{32}$$

The level set formulation can be done using the equation below:

$$\frac{\partial \phi}{\partial t} = spf_t\big(Im(x)\big) \cdot \alpha |\nabla \phi|, x \varepsilon \Omega \tag{33}$$

The regularization of the level set is done using the Gaussian filter (G_σ) as

$$\phi = \phi * G_{t_\sigma} \tag{34}$$

2.3.1.8 Medical Image Segmentation Based on a Hybrid Region Based Active Contour Model[27]

This is a hybrid algorithm based upon the local and global parameters, in order to deal with the issues of intensity in homogeneity. The energy function used in this approach is a weighted sum of local, global and regularization parameters. The curve evolution equation is derived by minimizing the energy.

The energy function is a hybrid energy function computed by:

$$E_t{}^{Hybrid} = \alpha E_t{}^{Global} + \beta E_t{}^{Local} + \omega L_t \tag{35}$$

Where $L(\phi)$ is the regularization term.

$$
\begin{aligned}
E_t{}^{Hybrid}&(\phi, m, n, u_x, v_x) \\
= \alpha &\int_{\Omega_y} \big(H_t\phi(y)(Im(y) - m)^2 + \big(1 - H_t\phi(y)\big)(Im(y) - n)^2\big)\, dx\, dy \\
+ \beta &\int_{\Omega_x} \int_{\Omega_y} B_t(x,y)\,(u_x - v_x)^2\, dx\, dy \\
+ \omega &\int_{\Omega_x} \delta\phi(x)|\nabla\phi(x)|\, dx \tag{36}
\end{aligned}
$$

The level set function can be minimized by using the equation as:

$$
\begin{aligned}
\frac{\partial \phi}{\partial t}(x) = {}& \alpha\delta\phi(y)[-(m-n)(2Im - m - n)] \\
& + \beta \int_{\Omega_x} B_t(x,y)\delta\phi(y) \\
& \times \left(\frac{(Im(x) - u_x)^2}{A_u} - \frac{(Im(x) - v_x)^2}{A_v}\right) dy \\
& + \omega\delta\phi(x)div\left(\frac{\nabla\phi(x)}{|\nabla\phi(x)|}\right) \tag{37}
\end{aligned}
$$

A_u represents the local interior area and A_v represents the local exterior area.

2.3.1.9 Region Based Segmentation in Presence of Intensity in Homogeneity Using Legendre Polynomials[30]

This approach of segmentation is based upon the Chan-Vese approach in which the energy function is represented as:

$$\varepsilon_t(\phi, c_1, c_2) = \int_\Omega |f_t(x) - c_1|^2 \, H_{t_\varepsilon}(\phi) \, dx$$

$$+ \int_\Omega |f_t(x) - c_2|^2 \left(1 - H_{t_\epsilon}\phi(x)\right) dx \qquad (38)$$

It improves the energy function $\varepsilon_t(\phi, c_1, c_2)$ by replacing the constants c_1 and c_2 by two smooth functions $c_1^m(x)$ and $c_2^m(x)$. These smooth functions are represented as a combination of some Legendre functions.

The energy function is given by

$$\varepsilon_t{}^m(\phi, A, B) = \int_\Omega |f_t(x) - A^T \mathbb{P}_t(x)|^2 \, m_1(x) \, dx + \lambda_1 \|A\|_2^2$$

$$+ \int_\Omega |f_t(x) - B^T \mathbb{P}_t(x)|^2 \, m_2(x) \, dx + \lambda_2 \|B\|_2^2$$

$$+ v \int_\Omega \delta(\phi) \frac{\nabla\phi}{|\nabla\phi|} dx \qquad (39)$$

The curve evolution is performed with the following equation:

$$\frac{\partial\phi}{\partial t} = [-|f_t(x) - A^T \mathbb{P}_t(x)|^2 + |f_t(x) - B^T \mathbb{P}_t(x)|^2]\delta_\varepsilon(\phi)$$

$$+ v\delta_\varepsilon(\phi) div\left(\frac{\nabla\phi}{|\nabla\phi|}\right) \qquad (40)$$

2.3.1.10 Segmentation of Regions of Interest Using Active Contours with SPF Function [1]

The proposed method aims at segmenting the image into any number of sub regions, and then step by step each region is considered. A signed pressure force (SPF) function is used in the level set formulation. The procedure keeps on dividing the sub regions iteratively and discards the outer regions till some stopping criteria is met. It does not require re-initialization.

The energy function is calculated by using the equation as given below:

$$E_{t_{proposed}} = \int_\Omega |Im(x) - c_1|^2 \, H_{t_\varepsilon}\big(\phi(x)\big) M_t{}^k(x) \, dx$$

$$+ \int_\Omega |Im(x) - c_2|^2 \left(1 - H_{t_\varepsilon}\big(\phi(x)\big)\right) M_t{}^k(x) \, dx \ (41)$$

The level set is regularized using the Gaussian kernel as

$$\phi^n = G_{t_\sigma} * \phi^n \qquad (42)$$

3   Results and Discussion

For the purpose of comparison a sample image from the ADIR dataset has been taken as shown below in fig 5 below. The ground truth for the image taken is as given in table 1. As per the ADIR dataset the image named P110536D, has 43 chromosomes and 2 noisy objects as depicted in figure. Out of 43 chromosomes 39 chromosomes are individual and 4 chromosomes are present as two clusters having 2 chromosomes each.

Table 1 Ground

Object Present	45
Noisy objects	2
Total Chromosomes	43
Individual Chromosomes	39
Cluster of Chromosomes	2

Fig. 5 Image P110536D from ADIR dataset truth for Sample Image

The sample image was segmented using the 8 level set based algorithms that has evolved over the years and the results of comparison based upon the segmentation results are shown in the table 2 below. The segmentation results have been compared for the parameters of total number of iterations, total number of objects segmented, count of individual chromosomes segmented, count of noisy objects segmented, count of cluster of chromosomes segmented, and finally accuracy is measured in terms of correctness of number of individual chromosomes segmented. Seeing the results of table 2 below the fastest method for level set segmentation is Variational B-spline level-set: a linear filtering approach for fast deformable model evolution.[8], the methods proposed by [26]&[36] are also quite fast as compared to others. But while implementing [36] in MATLAB environment it was quite slow, but is worked quite well in C++ environment. The segmentation results for the segmentation of the metaspread images were best with the [26], it was capable of segmenting the metaspread image with 100% accuracy, but this method has a limitation that it results in over segmentation in case of noisy object.

The method proposed by [10] is very slow and do not work well in case of objects which are lying close by, it segments them as a cluster of chromosomes. In addition to that the accuracy of segmentation is very low.

Most of the proposed segmentation methods based upon level set are enhancements of the [12]. It is quite good at the segmentation, is able to segment the nearby objects with good accuracy. The major drawback of this method is the speed. The segmentation accuracy is at par with the recently proposed methods. The method proposed by [24] is based upon [12], but was not able to segment the metaspread images, it is suitable for segmenting a single object only. It is slow and is heavily dependent upon the initial contour.

As per the literature survey the fastest of all the methods till the year 2008 was [36],as it is based upon the linked list data structure, but it was not able to work well while segmenting the metaspread chromosome images. It is very much dependent upon the initial contour. It is not able to segment the objects that are lying close to each other.

A B-Spline based very fast method has been proposed by [8], it is heavily dependent upon initial contour. It was not able to segment the objects lying close to each other. It is fastest of all the methods, but is not suitable for the segmentation of metaspread chromosome images.

Recently a method has been proposed by [30], it is uses Legendre polynomials, but this method is very slow. But it is able to segment the objects with good accuracy.

The method proposed by[45] based upon local and global segmentation is fast. Is not dependent upon the initial contour value and can segment the chromosomes with quite good accuracy. It is the near competitor of the best method from the selected methods in terms of speed and accuracy of segmentation. Form the comparison of table 2 below the best performer is the [26], which is able to segment the metaspread chromosomes with 100% accuracy. The only drawback of this method is that it results in over segmentation in case of very large noisy objects, as these large noisy objects have varying intensity values due to which this over segmentation results.

Table 2 Results of segmentation on application of chosen level set based algorithms

S No	Parameter	[10]	[12]	[26]	[24]	[36]	[8]	[45]	[30]
1	Segmentation results								
2	Speed	Slow	Slow	Fast	Slow	Very Fast	Very Fast	Fast	Slow
3	Number of Iterations	4000	3810	186	561	34	10	220	2178
4	Count of Objects segmented	23	41	47	1	23	33	41	40
5	Count of Noisy objects	3	2	6	1	1	1	2	1
6	Count of Individual Chromosomes	13	36	39	0	21	25	36	36
7	Count of Cluster of Chromosomes	7	3	2	0	1	7	3	3
8	Accuracy	33.3%	92.3%	100%	0%	53.8%	64.1%	92.3%	92.3%

Table 3 Comparative analysis of the chosen algorithms

S No	Parameter	[10]	[12]	[26]	[24]	[36]	[8]	[45]	[27]	[30]	[1]
1	Based upon	Geodesics curves	Mumford shah model	Gaussian Geodesic curves	Chan Vese&Yezzi	Approxima-tion of level set	B-Splines & Mumford Shah model	Geodesics curves and Chan Vese	Chan Vese , Linkton& Yezzi	Chan Vese	Chan Vese & C Li
2	Energy	Contour Based	Region Ba-sed	Localized region Based	Localized region based	Region Ba-sed	Region Based	Localized Region Ba-sed	Region Ba-sed	Localized Region Based	Localized Region Based
3	Speed	Slow	Slow	Fast	Slow	Fast	Fast	Fast	Fast	Slow	Fast
4	Evolution	Narrow Band	Narrow Band	Whole Domain	Narrow Band	Narrow Band	Whole Domain	Local & Global, its optional	Narrow Band	Narrow Band	Narrow band
5	Threshold Energy	Local	Global	Local	Local	Local	Local	Local	Hybrid	Local	Local
6	Re-initiali-zation	Yes	Optional	No	Yes	No	No	No	No	No	Yes
7	PDE	Yes	Yes	No	Yes	No	No	Yes	No	Yes	Yes
8	Energy Terms	1	2	3	2	2	2	2	3	3	2
9	initialization	Yes	No	No	Yes	Yes	No	No	Yes	Yes	Yes
10	Regulariza-tion Term	Yes	Yes	Yes	Yes	Yes	No	Yes	Yes	Yes	Yes

In the table 3 above the various level set based methods have been compared based upon the underlying implementation details. The parameters used for the comparison of the various models are :*Based upon, Energy , Speed, Evolution, Threshold Energy, Re-initialization, PDE, Energy Terms ,initialization, Regularization Term.*

4   Conclusions

In this work various level set based segmentation algorithms have been examined for their suitability for the segmentation of the metaspread images of the human chromosomes. The metaspread images suffer from intensity inhomogeneity and are therefore difficult to segment with the normal segmentation methods. The level set based methods are quite efficient at segmentation of the images with intensity in homogeneity. As they draw the intensity information around both sides of the contour. Ten algorithms have been studied that have evolved over a period of time for the segmentation of images. The best performer has been the minimization of region scalable fitting energy for image segmentation approach. It was able to segment the metaspread image with the same accuracy as that of an expert. It is very efficient when it comes to segment the objects that are lying quite close to each other. The only drawback is that it is comparatively slower than other available methods as it results in over segmentation. The selected method is the best candidate for segmenting the metaspread images of human chromosomes.

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