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G-Band Chromosome Segmentation, Overlapped Chromosome Separation and Visible Band Calculation

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ABSTRACT Chromosome segmentation and overlap removal plays a vital role in chromosome analysis. Genetic disorders are detected through chromosome analysis. Many methods for chromosome analysis were proposed and most of them require human intervention at some point. In this paper, a novel idea to extract the visible bands in human chromosomes are explained, chromosome contour is obtained using Multi-object Geodesic Active contour (MOGAC). Hypothesis based chromosome segmentation for overlap separation is performed. The initial step is to segment and separate the non-overlapped chromosomes from metaphase spread chromosome image using MOGAC. This algorithm improves the segmentation accuracy for multiple object segmentation. For overlapped chromosomes, an automatic geometry separation algorithm is developed. This helps in separating the overlapped chromosome images. The main step is to identify the cut points on the image. With the obtained cut points, hypothesis lines are drawn over the overlapped region. This line shows the separation region. Hypothesis verification is done for obtaining the proper disentangled chromosome images. Vector quantization is used for band calculation in L^*a^*b colour space. The accuracy of the algorithm is about 96%. Centromere identification is performed by distance transformation. Based on band measurement and centromere position, homologous chromosomes are obtained.

INTRODUCTION

Chromosome image analysis is an important process in the field of cytogenetic to identify the chromosome abnormalities. Karyotyping (Speicher et al. 1996) is a process used to analysis these chromosomes for different genetic problems like Down syndrome, Turner syndrome, etc. So many techniques were proposed for karyotyping in image processing. Karyotyping analysis is an important screening and diagnostic procedure for detecting several genetic diseases and chromosomal anomalies. A chromosome anomaly reflects a typical number of chromosomes or a structural abnormality in one or more chromosomes. The numerical anomaly is a variation in the number of chromosomes. Structural anomaly is the breakage and loss of a portion of chromatid arm or a reunion of the arm at different location on the same chromosome or on a different chromosome. The initial and vital role to attain the accurate karyotyping is by segmenting the chromosome images. Normal translocation based labelling method (Schrock et al. 1996) for M-fish chromosomes are analysed. Fuzzy c-means based segmentation (Mahalakshmi et al. 2013) provides the background correction and performs the segmentation .Genetic algorithm based segmentation provides a better search and optimization technique and contour method as used to identify the boundaries. Watershed segmentation (Petros et al. 2010) is applied to M-fish chromosomes for segmentation process. This method performs better than other method but faces over segmentation problem. K-means segmentation is an initiative to other segmentation methods. In which the cluster deformation is high compared to watershed segmentation. This leads the data inaccuracy in the segmentation. Fuzzy subset based segmentation performs segmentation based on shape breakdown, from which many automated and semi-automated segmentation and karyotyping methods are proposed earlier. Automated and semi-automated karyotyping of chromosomes (Carothers et al. 1994; Leady et al. 1980) is studied. Chromosome analysis is done using the biometrics to find out the bands in the chromosomes (Somasundaram et al. 2013). Computer based karyotyping initially introduced based on

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the virtual reality on the shapes. Heuristic edge linking method separate overlapped chromosomes using connected in component path. This method is inefficient for curved overlapped chromosomes due to the link lost connection between chromosomes. Curved chromosomes are straightened for better analysis of the chromosome images (Devaraj et al. 2014), Automatic segmentation of overlapped chromosome using intersecting points analysed. This method inefficient when the chromosome having more than one overlapped chromosomes. In this paper, MOGAC segmentation and Hypothesis analysis applied to chromosome segmentation for overlapped, non-overlapped chromosomes and comparison between the previous segmentation methods with various parameters are analysed.

Organizations of this paper as follows, section 2 provides MOGAC segmentation and its procedures. Section 3 discusses the hypothesis verification. Section 4 discuss the centromere identification, section 5 present the results of proposed method for overlapped chromosomes and non-overlapped chromosomes. The conclusion of the paper is presented in section 6.

PROPOSED METHOD

Figure 1 shows the work flow of chromosome segmentation. In this segmentation process, the Meta spread chromosome image is converted into Gradient components. The image is initially converted to Gray scale. MOGAC algorithm segment and separate the overlapped and non-overlapped regions. Overlapped regions separated through hypothesis analysis as explained in section III.

Algorithm steps,

(i). Input a metaspread chromosome image
(ii). MOGAC algorithm for chromosome segmentation (Section 2.1)
(iii). ROI selection of overlapped chromosomes
(iv). Hypothesis analysis for chromosome separation (section 3)
(v). Colour space conversion for band identification (section 3.3)
(vi). Vector quantization network for band selection (section 3.4)
(vii) Area Based band count in each chromosomes.

MOGAC Algorithm

MOGAC algorithm is a level set progress algorithm, (Blake c Lucas et al. 2012). Gray scale converted image is taken as input to MOGAC. Let Gray scale image is considered as I. then,

$1: \Omega \rightarrow R, \Omega \subset R^d$, where d $\varepsilon \{2,3\}$.

For each N-region equation is represented as,

$$\psi_n : \Omega \rightarrow \mathrm{R} \text{ and } \eta \varepsilon £ = \{1,2,.....,N\}$$

The above equation includes signed field & labelled regions.

The Label function is given as $X: \Omega \rightarrow £$ and unsigned distance field as $\psi: \Omega \rightarrow \mathrm{R}$:

$$\psi(x) = \min_n | \varphi_n(x) | \quad (1)$$

$\varphi_n(x)$ is computed from $X(n)$ and $\varphi(x)$ at the boundary is given as,

$$\Lambda_n = \{x \mid \exists\ y \varepsilon N(x) \text{ s.t. } \sigma_x(x) \neq \sigma_n(y)\} \quad (2)$$

Where $N(x)$ is the 2d connected neighbourhood of pixel x and $\sigma_n(x)$ is,

$$\sigma_n(x) = \begin{cases} -1 & X(x) = n \\ 1 & otherwise \end{cases} \quad (3)$$

The reconstruction of partially level set is given as,

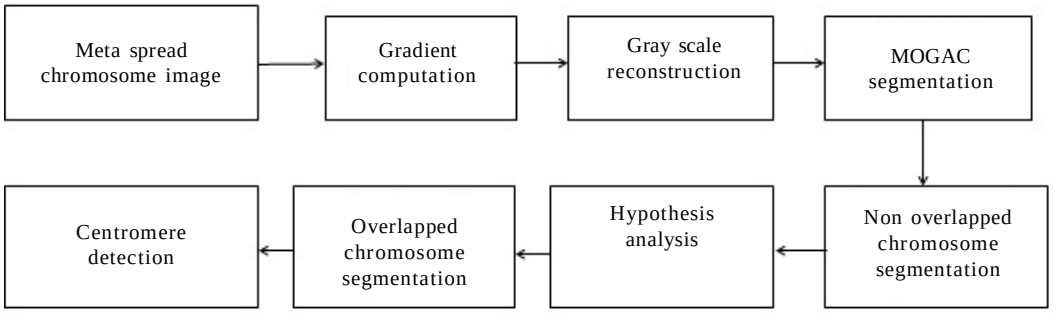

Fig. 1. Flow chart of the proposed method

$$\psi_n(x) = \sigma_n(x)\,\psi(x) \quad (4)$$

The differential equation for N level set is given as,

$$\frac{\partial \psi_n(x.t.)}{\partial t} = f(x,t)\,\delta(\varphi(\xi,\tau)), \quad (5)$$

Where, $f(x,t) \epsilon R^N$ is the function used to represent speed and $\delta(.)$ is an NxN diagonal matrix.

$$\varphi(x, t+\Delta\tau) = \varphi(x, t) + \Delta t f(x, t)\partial(\varphi(\xi,\tau)), \quad (6)$$

The level set is obtained by each iteration with small incremental changes in 't' as explained in equation 6. After computing all the updates in each level sets, the result are stored in the label and unsigned regions of chromosome images. When $\varphi_a(x) < \varphi_b(x)$, the updation in the label and unsigned regions for n labels are given as, $x \epsilon \Lambda_n$

$$\chi(x, t) = \begin{matrix} b \\ a \end{matrix} \quad \begin{matrix} \varphi_a(x,t) < \varphi_b(x,t) \text{ and } b < a \\ \text{otherwise} \end{matrix} \quad (7)$$

and

$$\varphi(x,t) = \frac{1}{2} \left| \varphi_a(x,t) - \varphi_b(x,t) \right| \quad (8)$$

Level set progress consists of two algorithms are evolve and rebuild. In which evolve is used to evaluate the process and evolve is a straightforward to parallelize the process. In which Ω is dependent on only the 2d connected neighbourhood around pixel. The computational complexity is O (M^d/P) for M^d pixels and P processing units for distance of L= 3 pixels.

The algorithm for evolve and rebuild are considered for obtaining the ROI by the above algorithm the ROI for overlapped and non-overlapped chromosomes in the meta spread images are identified. From this the overlapped images are taken for further study. The cut points and hypothesis lines are obtained for separation process. The identification of cut points and hypothesis lines are explained below.

Detection of Hypothesis Lines

The cut points are detected based on the computational geometry (Karvelis et al. 2010) on the image boundary. The computational geometry mainly deals with detecting the curvature function. The curvature function helps in finding the cut points on the image that helps in separating the overlapped region. The curvature function is defined as the change in the rate of the curve slope with respect to its length. The curvature function is represented as

$$y = \frac{d\varphi(s)}{ds} = \frac{1}{p} = \frac{\bar{\bar{y}}}{\left(^{-2}_{y+1}\right)^{\frac{3}{2}}} \quad (9)$$

Where $\bar{\bar{y}}$ and $\bar{y}$ represents the second and first order derivative of the curve function. $\varphi(s)$ is the slope of the curve. γ is curvature function. P is called the radius of the curve function. The curvature function helps in determining the boundary points. The boundary points over the overlapped region are the cut points. The proper cut points are obtained as the boundary is smoothened by polygon approximation. The cut points are obtained with the nearest neighbourhood connected cut point candidate. The overlapped region can have two parallel cut lines. The possible cut lines are detected using the neighbourhood pixel functions. The cut lines helps in determining the separation of the overlapped portions in the image. An initial seed point or the cut point is identified. A search direction is introduced to obtain the nearest next cut points. The search direction is updated to find the next upcoming neighbour points to achieve a proper cut line called as pale path for separating the overlapped region. Let B be the binary image. The cut points on the image are C_1, C_2,....C_3. Where C is the cut points and N is the total pixel points. The cut points are obtained by a curvature function where the maximum points are considered as the cut points and are plotted on the image. The cut lines are drawn by the Euclidean distance function. $d=\sqrt{(x-x_1)^2 - (y-y_1)^2}$ points of $x-x_1$ are the cut points and $y-y_1$ end points of the image. With this the overlapping problems get a solution and the separated images are obtained. After the separation is obtained if the chromosomes are identified as curved images straightening is performed.

Centromere Identification

Centromere is a part of chromosome and helps in linking the sister chromatid. Centromere position helps in homologous chromosome classification. The centromere positions are identified by several steps. The initial input is taken as an individual chromosome image. The second step is the conversion of input image to binary image. In binary image if there is any opening that can be closed by filling process so a good binarized image can be obtained. The explanation of the above steps is already done

for segmentation operation. The third step is the Euclidean distance transform. In this medial axis is identified in the image for centromere location. For extracting the medial axis a distance transform or Euclidean transform is considered. Finally the centromere of the chromosome is obtained. With this the segmentation output is compared and the homologous chromosome is identified.

Centromere Extraction

The centromere is identified by a simple distance transformation technique. The distance transform provides a metric or measure of the separation of points in the image. It computes the Euclidean distance transform of the binary image. For each pixel in the image, distance transform assigns a number that is the distance between that pixel and the nearest nonzero pixel for any dimensional image. The input image is initially converted to binary image. The image is then processed by distance transform technique. A profile is drawn perpendicular to in the transformed image which gives a plot through which the location of centromere can be identified. Two different type of profile is considered in this work namely density profile, shape profile. Density profile is the average grey scale value of all perpendicular line across the medial axis of a chromosome image. It is computed by the given formula:

$$D(x) = \sum_{i=1}^{n} \left[g_i(x) / n \right] \quad (10)$$

Where $gi(x)$ is the Gray value of each pixel in a perpendicular line and n is the number of all pixels in each perpendicular line. The computer scheme applies a median filter to reduce possible impulses and noise in the density profile. A shape profile gives the weighted width for all perpendicular line across the medial axis of a chromosome image. It is defined by the formula:

$$s(x) = \sum_{i=1}^{n} \left[g_i(x) \times d_i(x)^2 \right] \sum_{i=1}^{n} d_i(x)^2 \quad (10a)$$

Shape profile corresponds to the sum of the product of the grey scale value $gi(x)$ and its corresponding Euclidean distance $di(x)$ away from the medial axis of the perpendicular line, divided by the sum of the distance (Wang et al.2008). With the shape and density profile the centromere position is calculated. The segmented output image is taken and verified with the outputs obtained for the homologous identification. The homologue chromosome images are identified by the centromere position. The original chromosome image centromere position is identified and the same algorithm is used for the separated chromosome image.

Band Calculation

In chromosome images, band and non-band are having the minimum colour illumination in different ranges. Work flow is shown in Figure 2. Differentiating the band and non-band with colour difference is a very difficult process. Chromosomes have same colour space occurrence and the variations between Non-band (light black) and band (dark black) are minimum. So colour space is converted from RGB to L^*a^*b is done. L^*a^*b is a linear colour space with minimum range of values between 0 to 1. Direct conversion transformation not having any useful meaning to convert RGB to L^*a^*b. This ANN network is trained to get all possible color variations by 288 different color in IT8 chart (Mathews 1992). This provides the differential internal energy change values in color less than 1.The generated internal energy values helps in classifying the band and non-band regions.

Neural Network Model

In color space conversion neural network used to normalize values in between the range of 0 to 1. This net had parameters of one neuron for each colour in the input layer. Finally it preserves three neurons in input layer and 3 neurons in output layer for each colour. Only one hidden layer, for train the neural net carried with two or more layers when the number of neurons in the layer is not constant. In ANN, reference mean value and test images are used for dimension reduction, to analyse the performance of inaccuracy in training and to stop in optimal condition early stopping in the Mat lab neural network tool was used. In our ANN model provide better results for conversion ($L^*a^*b^*$) and the calculation carried out within 59 Sec.

$$y_1 = \frac{yi \quad ymin}{ymax - ymin} \quad (11)$$

In (11), y_i – Normalized original value in the specified range w.r.to input variable, y_{min} – Minimum normalized value w.r.to input variable, y_{max} – Maximum normalized value w.r.to

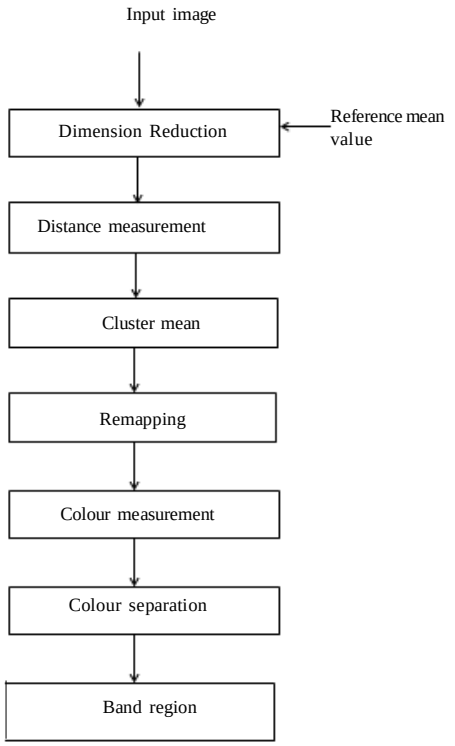

Fig. 2. Local band calculation using Dimension Reduction

input variable. For color correction gamma model,

$$R_y = \frac{(R + a_1)^y}{a_2} \quad (12)$$

$$G_y = \frac{(G + a_1)^y}{a_2} \quad (13)$$

$$B_y = \frac{(B + a_1)^y}{a_2} \quad (14)$$

Mathematically Color space conversion model,

$$\hat{L}^* = \begin{cases} 116 \left(\frac{Y}{Y_n}\right)^{\frac{1}{3}} - 16 & \text{if } \frac{Y}{Y_n} > 0.008856 \\ 903.3 \left(\frac{Y}{Y_n}\right)^{\frac{1}{3}} & \text{if } \frac{Y}{Y_a} \leq 0.008856 \end{cases} \quad (15)$$

$$\hat{a}^* = 500\left[\left(\frac{X}{X_n}\right)^{\frac{1}{3}} - \left(\frac{Y}{Y_n}\right)^{\frac{1}{3}}\right] \quad (16)$$

$$\hat{b}^* = 200\left[\left(\frac{Y}{N_n}\right)^{\frac{1}{3}} - \left(\frac{Z}{N_n}\right)^{\frac{1}{3}}\right] \quad (17)$$

$$\begin{bmatrix} L \\ a^* \\ b^* \end{bmatrix} \begin{bmatrix} M_{11} & M_{12} & M_{13} & M_{14} & M_{15} & M_{16} & M_{17} & M_{18} & M_{19} & M_{1,10} \\ M_{21} & M_{22} & M_{23} & M_{24} & M_{25} & M_{26} & M_{27} & M_{28} & M_{29} & M_{2,10} \\ M_{31} & M_{32} & M_{33} & M_{34} & M_{35} & M_{36} & M_{37} & M_{38} & M_{39} & M_{3,10} \end{bmatrix} \begin{bmatrix} R \\ G \\ B \\ RG \\ RB \\ GB \\ R^2 \\ G^2 \\ B^2 \\ 1 \end{bmatrix} \quad (18)$$

Above equations (15), (16), (17) condition for conversion of colour space, equation (12), (13), (14) provide correction factors. Equation (18) is used for matrix transformation.

Region-based Band Selection

In chromosome images, colour spread density is more when it is compared to other colour images because of light reflection and refection in the images. Classification of Band is more complicated to vision. In such condition, band selection is needed to make a reference seam which is easily separated from other seam regions. A band region is taken as reference seam because it is having dark black visualization region which is more comfortable to detect. From the non-band region, different Region of interests is selected randomly. These selection consist region 1 as background region, region 2 as dark Black region, region 3 and region 4 are two unknown reference colour regions accordingly. Background regions 1 not consider because of its unique color distribution. Initially the color space are converted to L^*a^*b colour space. In which, each region as approximated and compared with a reference region, each region is separately approximated with its RGB mean values w.r.to L^*a^*b, for each region 3 values are calculated. It is for region 1 mean value as indicated in Table 1 as [0.3317 0.2525 0.1966]. This process is extended to all the three regions.

RESULTS AND DISCUSSION

In this, experiment carried out in MATLAB 2012a version, windows 7 64-bit operating system. Results of the proposed method in chromosome segmentation as implemented in Meta spread image. Figure 3a shows the obtained contour image. The region boundary is obtained after the completed iteration of the level set algorithm. The boundary is obtained by consider-

Table 1: Colour space converted values from RGB to L ‘a’b

(:,:,1) =								(:,:,2) =							
0.3804	0.3922	0.3765	0.3373	0.3059	0.2824	0.2706	0.2627	0.3137	0.3176	0.3020	0.2627	0.2314	0.2078	0.1961	0.1882
0.3804	0.3922	0.3765	0.3373	0.3059	0.2784	0.2706	0.2588	0.3137	0.3176	0.3020	0.2627	0.2314	0.2039	0.1961	0.1843
0.3882	0.3961	0.3804	0.3412	0.3059	0.2745	0.2667	0.2588	0.3137	0.3216	0.3059	0.2667	0.2314	0.2000	0.1922	0.1843
0.3922	0.4000	0.3843	0.3451	0.3137	0.2706	0.2627	0.2549	0.3176	0.3255	0.3098	0.2706	0.2392	0.1961	0.1882	0.1804
0.4118	0.4157	0.3882	0.3529	0.3255	0.2863	0.2784	0.2706	0.3373	0.3294	0.3020	0.2667	0.2392	0.2000	0.1922	0.1843
0.4118	0.4078	0.3804	0.3451	0.3176	0.2824	0.2824	0.2784	0.3373	0.3216	0.2941	0.2588	0.2314	0.1961	0.1961	0.1922
0.4157	0.4000	0.3686	0.3333	0.3098	0.2824	0.2941	0.2941	0.3294	0.3137	0.2824	0.2431	0.2196	0.1882	0.2000	0.2078

(:,:,3) =															
0.2510	0.2588	0.2431	0.2039	0.1725	0.1529	0.1412	0.1333	0.3317	0.2525	0.1996					
0.2510	0.2588	0.2431	0.2039	0.1725	0.1490	0.1412	0.1294	0.2420	0.2091	0.1622					
0.2549	0.2627	0.2471	0.2078	0.1725	0.1451	0.1373	0.1294	0.4599	0.4319	0.3574					
0.2588	0.2667	0.2510	0.2118	0.1804	0.1412	0.1333	0.1255								
0.2784	0.2745	0.2471	0.2118	0.1843	0.1490	0.1412	0.1333	0.3317	0.2420	0.4599					
0.2784	0.2667	0.2392	0.2039	0.1765	0.1451	0.1451	0.1412	0.2525	0.2091	0.4319					
0.2745	0.2588	0.2275	0.1882	0.1647	0.1412	0.1529	0.1569	0.1966	0.1622	0.3574		0.723			

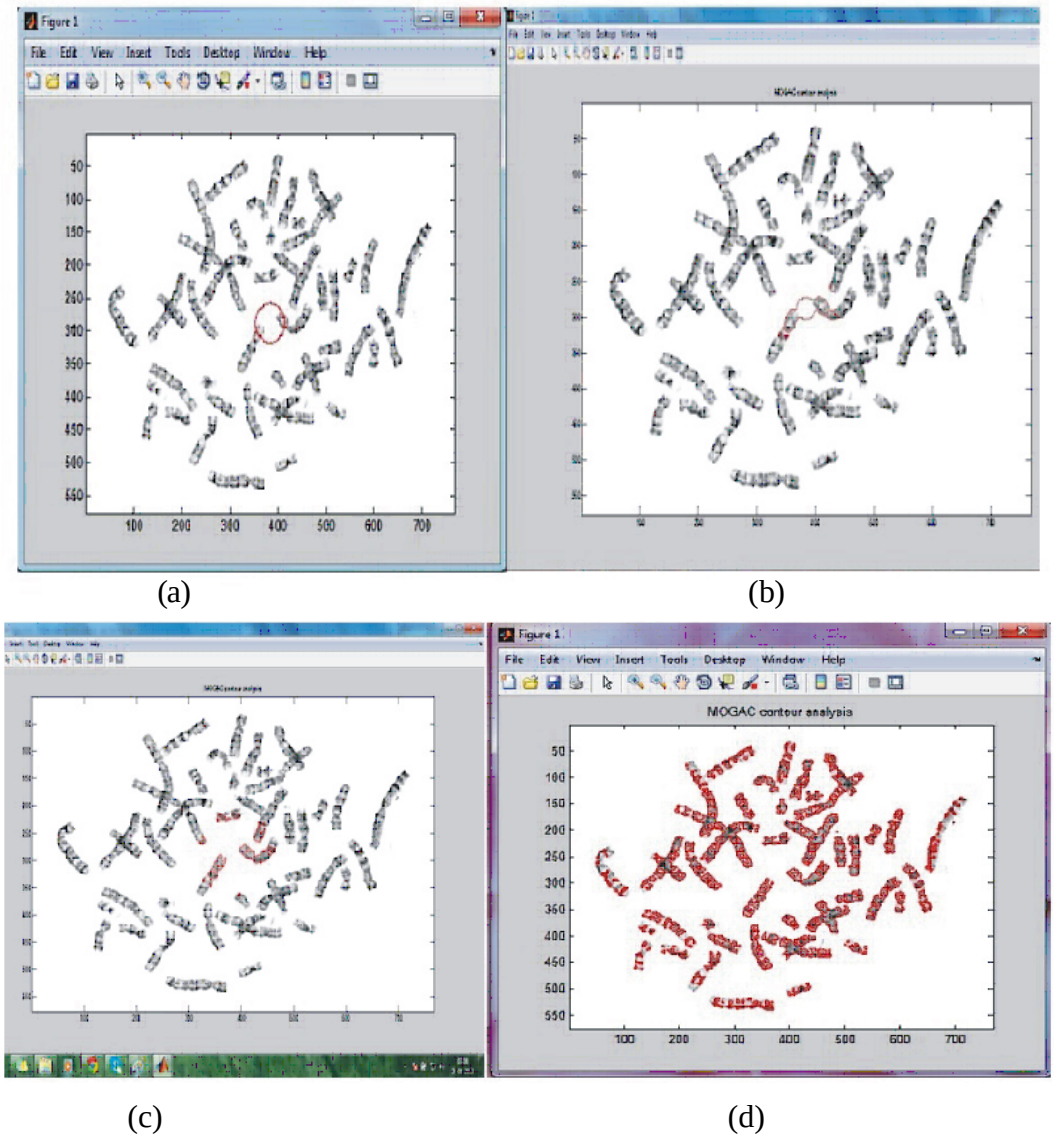

Fig. 3. (a) Initial contour region generation, (b) iteration based contour growing (c) Segmentation of chromosome with contour region (d) MOGAC Segmented image

ing the proper search direction. The search direction is obtained using 2d connected component as shown in Figure 3b.

Figure 3c, shows the individual non overlapped chromosomes are segmented with higher accuracy. Figure 3d provides the segmentation of the Meta spread chromosome image. In which, touching chromosomes are automatically segmented and the overlapped chromosomes are segmented with the connection of two chromosomes. In this case, hypothesis based analysis is performed in the overlapped chromosome for the accurate separation in the overlapped chromosomes.

The overlapped chromosomes of the Meta spread is considered as the Region of interest (ROI).Morphological operations are carried out on the overlapped chromosomes, concave and

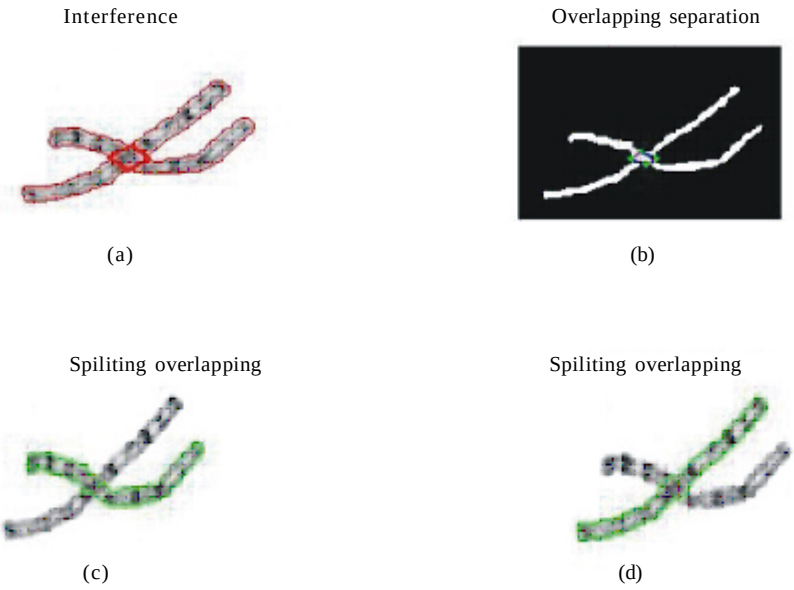

Fig. 4 (a). Inference region in overlapped chromosome, (b) Overlapping separation, (c) Segmentation of chromosome 1, (d) Segmentation of chromosome 2

convex point are calculated. Higher and lower corner intensity points are considered as concave and convex points. Based on those points the contour plot is generated.

Figure 4(a) shows the inference region between the two overlapped chromosomes. From the inference, the overlapped region points are validated as shown in Figure 4(b). After analysis in the overlapped region, each individual chromosome is separated from the overlapped region shown in Figure 4(c) as chromosome1 and Figure 4(d) as chromosome 2. These processes applied to all overlapped chromosomes, separation of individual chromosomes are done. Analyses of proposed work with other segmentation methods are given in Table 2. In which, proposed method tested with 300 non overlapped chromosome images and 220 normal chromosome images which as selected from metaphase spread based on region of interest. This is analysed with maximum likelihood technique (Schwartzkopf et al. 2005), watershed transform and k-means clustering, fuzzy c-means clustering techniques as shown in Table 2.

Selection of band and Non-band regions in the chromosome are done. Each region provides the test values as input for vector quantization in order to generate the colour space values. In this, selection of dark band region, non-band

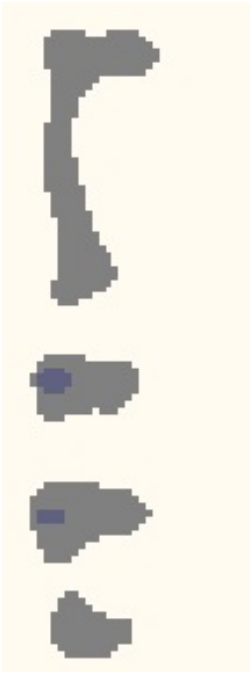

Fig. 5. Local bands in chromosome

Table 2: Comparison of various segmentation methods

		Water shed Transform	*K-means algorithm*	*Fuzzyc-means*	*Proposed method (MOGAC)*
Segmentation and Separation Accuracy	Accuracy (%)	80.4	76.5	91	94.6
	Selectivity	0.81	0.81	0.80	0.95
	Specificity	0.79	0.79	0.78	0.89
	Single object	0.88	0.90	0.86	0.95
	Oversegmentated object	4.45	3.45	5.09	0.5
	Non overlapped images	300	300	300	300
	Overlapped images	220	220	220	220

region, reference regions are necessary to identify band region. Figure 5, shows the vector quantised regions for the band regions and also Figure 5 shows the total number of bands present in a chromosome.

CONCLUSION

This paper presents MOGAC based segmentation technique for metaphase spread chromosome images. Hypothesis curvature based method as additionally used to segment and separates the overlapped chromosomes. A new boundary region to the labelled region based updation method is proposed in order to increase the accuracy and reduce the time complexity. Proposed method automatically segments the touching chromosomes during the iteration. So complexity in overlapped chromosome segmentation is reduced. Vector quantization is used to calculate the band for irregular banding chromosomes. This method has low complexity to work in all system software and Personal computers.MOGAC and Vector quantization are more suitable for karyotyping systems in order to increase the accuracy.

RECOMMENDATIONS

Accuracy in the identification of local bands can be further developed with newer segmentation algorithms using various classifiers. Identification of centromere position and band calculation can help in identification of homologue chromosomes for better classification.

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