New algorithm for separation overlapping & touching chromosomes

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Abstract. The karyotyping technique is importance in the diagnosis of genetic diseases of humans, including the diagnosis the genetic disorders of prenatal and cancer. In order to obtain the karyotype system for the metaphase images of chromosomes using the image processing. Assembling of human chromosomes pairs from the metaphase image will pass in several stages which are segmentation (single chromosome, overlapped and touching chromosome), feature extraction, matching and classification. In this study has been focus on the separation of touching and overlapped. The problem of separation touching and overlapped chromosome was solved using the convolution mask and with a help the morphological thinning and contour. The separation of touching and overlapped chromosome is according on finding cut points. The thinning of the image is obtained which helps to identification the Region of Interest. The contour of the image is obtained which helps to explain the entire shape of the image and find out the cut points for separation between chromosomes. In this study an efficient algorithms was used to separation the cluster of touching and overlapping chromosomes. The algorithm of separation touching and overlapping was implemented by using sequential stages (initially, segmentation from metaphase image, convert to the binary image, extract thinning, using mask with 9 *9, extract contour, using mask 7*7,identified the cut points and finally, separate touching or overlapping chromosome). This algorithm capable to isolate a cluster of touching chromosomes and a cluster of touching and overlapping chromosomes.

Keywords: karyotyping, chromosomes, image processing, touching and overlapping.

1. Introduction
Chromosome karyotyping is a necessary function in genetics and is typically performed in clinical and genetics laboratories and can be utilized in the diagnosis of genetic disorders [1]. The karyotype is consists of the identification, classification and presentation of the 23 pairs of the chromosome from one cell of normal human either by drawing or by photography employing a microscope in a single image. The pairs of chromosomes are classified as 22 autosomal pairs and a single pair of sex chromosomes.
for determinate the human gender either male or female [2]. A karyotype is required to assign each chromosome to one of 24 classes [3]. The karyotyping process, that is typically done manually via somebody's expert is a difficult and time consuming mission [4]. The process of manual karyotyping is typically administered by expert clinicians, who at the start identifies every chromosome within the image using a hierarchy chromosome identification, and finally, expert information is used to display images, determine the chromosomes, cut and place them in their fixed positions in the karyotype and classifies them into smaller teams (groups) [5]. The chromosomes are non-rigid bodies that contain the hereditary info of an individual. The metaphase image of chromosome spread contains on the chromosomes, however these chromosomes aren't similarity bodies, they will either be single chromosomes, they will be bent, or may be touching one another, or maybe is also overlapping and so forming a clustering of chromosomes. The extraction of the single chromosomes from these touching and overlapping chromosomes is a terribly tedious processing [6]. Automated karyotyping is a difficult process due to the non-rigid nature of the chromosome [7]. In the current time, there exists software system perform the karyotype that extracts chromosomes from microscope pictures of cellular nucleous in metaphase case, and then classifier them later. This method is being more and more automated. However still some processes require minimal human intervention, so increase time for process, and the possibility of error [8]. Has being captured an images of the G-banded metaphase spreads by using the camera connected to the microscope. The resolution and clarity of the acquired chromosome pictures relies on the goodness of the automated abnormality discovery systems. The pre-processing techniques are applied on the taken image to enhance the quality of chromosome images. An example of these techniques are noise removal technique and contrast improvement techniques [9]. A segmentation technique is employed for the separation of single chromosomes from the metaphase image. Then will be extracted many of features from every chromosome is segmented and based on these features the pairing and classification are done [10].

As chromosome images are non-rigid in nature shape variability is very high may be have on the bent, single, touching and overlapping chromosomes. So, the algorithm to separate the overlapping chromosomes must necessarily be independent of the shape of the cluster. In this paper proposed algorithm to separate overlapping and touching chromosomes from the metaphase image. This problem was solved using the convolution mask and with a help the morphological thinning and contour of the overlapped or touching chromosome. This it worked by follow the algorithm in figure 1.
2. Related work

In Somasundaram, Devaraj, and VR Vijay Kumar, 2014 [11] the work mainly focuses on separation of overlapping chromosome images. The separation of overlapped chromosome images are acquired by the following procedure: 1) The overlapping chromosome image is obtained from segmentation process, 2) this image is converted into binary images by using threshold techniques, 3) Morphology operations are used for getting best a binary image. This first three steps place under pre-processing process of image. By using thresholding method the binary image is obtained. The morphology operations are applied in order to fill the holes on the result of threshold to induce good for produce correct segmentation. 4) Contour of the binary image is applied to identify the boundaries, 5) Cut points are specified and 6) cut lines are drawn on the overlapped region and in the end the image of overlapping chromosome is separated. A disadvantage of this paper, the original separate chromosome images are determinate based on the length and the area of the image and the homology determination is done based on the chromosome centromere location by using distance transformation, without taken considered to the overlapped region and banding information of the overlapping chromosomes. The authors Hu, R. Lily, et al, 2017 [12] in this paper, applied neural network-based image segmentation to the problem of distinguishing between partially overlapping human chromosomes. This search is used several steps to separate overlapping chromosomes. Initially, adaptive thresholding of gray image and labeling of connected components of the binary image to obtain on the single chromosomes and touching or overlapping chromosomes. The overlapping chromosomes are isolated and its contour is extracted. Remarkable points are found from contour, crossing domain can be found from four points then used to isolate the different parts of two crossing chromosomes. In this paper, is builted a dataset for semantic segmentation using thousands of semi-synthetically generated overlapping chromosomes, to create a segmentation solution to resolve overlapping chromosomes. where, the system produced in this paper cannot operate on the entire microscope images. Somasundaram, D., et al, 2014 [13] in this search a new idea was presente for discover the bands of human chromosome. where hypothesis in this paper is started from metaphase image acquired and segmentation image for obtain on the single and overlapped or touching chromosomes. The segmentation of single chromosomes from metaphase image was done by using MOGAC (Multi-object Geodesic Active contour). The separation of overlapping chromosomes was done based on the develop of an automatic geometry separation algorithm and which was perform based on the determinate the cut points of overlapped chromosome image. The cut points were identify by using computational geometry that deals with curvature function where this function was helped to detect the cut points of overlapped image. Madian, Nirmala, K. B. Jayanthi, and S. Suresh, 2018 [14] in this search was presented an automatic algorithm for isolated the overlapping and touching chromosomes from metaphase image. The algorithm used in this paper was pass in several phase which are (performing image binarization for chromosomes image input, extraction the contour image, compute the curvature function, locating concave points, Hypothesis analysis). The metaphase image of chromosome should be segmented for obtain on each object in the image which include the single and touching or overlapping chromosomes. To perform the segmentation process, initially the image must be converted by binary image, was use for that is the thresholding method. To obtained on the objects of image, was use the contour and the contour extraction has been help in separation the overlapping image. The interesting points (concave and convex points) has been calculated by using the curvature function. The intersection points has been computed according on the measurement of area, distance between the pixel points, angle. The separation of overlapping and touching chromosomes was obtain by using hypothesis analysis which depend on the compute Minset (the meaning of Minset is the Interconnection of few subset and compliment of rest of the subset for a region). The disadvantage of this search is compute the intersection point based on the curvature function, which has some complex. Madian, Nirmala, 2014 [15] in this search was focus on a create an algorithm for segmentation of touching chromosomes and overlapping chromosomes. The algorithm was include of several stage which started from acquire the RGB original image of metaphase chromosome, convert the RGB
chromosome image into gray scale image as output, convert the gray scale image into binary image by using thresholding method, extraction contour image from binary image for identify each object in image, apply the curvature function for identify the intersection points (concave or convex point). The separation of overlapping and touching chromosomes was obtain by using hypothesis analysis which depend on the compute Minset (the meaning of Minset is the Interconnection of few subset and compliment of rest of the subset for a region). The disadvantage of this search is a separation of overlapped chromosomes based on the an overlap minset without considering the banding information. Munot, Mousami V., et al. 2013 [16] this paper, proposed automatically algorithm for discovery intersection points in a group of overlapping chromosomes. To identify the intersection points (cut points), first step towards automated obtained of overlapping chromosomes from the metaphase. The intersection points were determinate based on the computational geometry of the boundary pixels. The algorithm needs further enhancement in cases where, the region of overlap was along the centromere of both the chromosomes.

3. Methodology
in this paper, builded an the proposed system for separation of touching and overlapped chromosome. The metaspread chromosome images are considered where the chromosome images are touching and/or overlapped to each other. These touching and overlapping chromosome images should be initially segmentation from metephase chromosome image to extract the single chromosome (chromosome object detection has obtained by using Region-Growing algorithm), feature extraction the features (length, centromere position, long arm and short arm). Then finally, classification.

4. Pre-processing
Usually a pre-processing step is very important to perform improvement in the next stages, Where this phase helps to improve the image and eliminate of any noise. In this article, Histogram equalization has been used to enhance the contrast and improve the image quality [17]. The result of Histogram equalization is placed in figure 2.

5. Chromosome Object Detection
In order to analyze the chromosomes, chromosomes objects are segmented from metaspread images and arranged in order to form a karyotype [18]. Extracting the chromosome from G-band metaphase images and compute the impotent features. Chromosome object detection must be considered carefully for splitting objects from the image background, convert to the binary image and region growing methods were implemented. In binary images, the concept of objects equals the concept of connected components. Therefore, through traditional algorithms, before computation the basic shape features of objects in a binary image, will usually need to perform labeling the related components to distinguish.
the different objects in the image [19]. Thresholding is the simplest method of image segmentation which can be used to create binary images from a gray scale image. The advantage of acquisition a first of a binary image is that it reduces the complication of the data and facilitate the process of recognition and classification [20].

The thresholding operation is a gray value remapping operation defined by:

\[
\text{If } (I(x, y) < T) \\
\text{If } (I(x, y) = 0) \\
\text{else} \\
I(x, y) = 1
\]

Where \(I(x, y)\) is an intensity value of the image pixel compared with a global threshold [21]. The figure 3 is demonstrate the result of the apply binarization method.

Once the binary image is obtained, the next step assign each object with special label. In this article the region growing method is used for this purpose. Region growing approach is that initial set of small areas are iteratively merged based on similarity constraints [22] as show in figure 4.

![Figure 3. Show the result of image.](image1.jpg)

![Figure 4. Labeling each chromosome object using region growing method.](image2.jpg)

6. The Separation Overlapped Chromosomes Are Done By Following Steps:

6.1 Stage 1:-

The overlapping chromosome image should be initially segmented from G-band metaphase chromosome image. The segmentation process performs using converted the image into binary and implemented the region growing. Thus the single chromosomes and overlapping or touching chromosomes are extracted. The algorithm is receiving the overlapped chromosomes image as input for it. show the figure 5.
6.2 Stage 2:-
In order to identification the intersection point of overlapped chromosome initially, extracted the thinning of binary overlapped chromosome image, show figure 6.

![Figure 6. Thinning image of overlapping chromosomes.](image)

6.3 Stage 3:- Calculate The Intersection Point And Identification The Region Of Interest.
Intersection point is important point in this algorithm for identified the Region Of Interest (ROI) on the overlapping region and calculation the four cut points that used to separate overlapped chromosomes. For that, performed some operation for calculate the intersection point, meaning with ROI is showed in the figure 7.

![Figure 5. a Original overlapped objects image, b: binary image.](image)
After extracted the thinning, the intersection point of thinning image was obtained by using two mask, the first mask with size 9*9 and the second mask with size 3*3. The intersection point detection to thinning of overlapped images has been following the next steps:

a) Initially, scan the thinning image and check each pixel each time whether it belong to the background (back pixel) or to the line of thinning (white pixel), if this pixel is belong to the line thinning (255).

b) passing the mask with 9*9 on the thinning image and compute the neighbors for visited pixel in the mask. Show the figure (8), when the number of neighbors of current pixel exceeds or equal the number 13. Thus, this pixel is candidate for assign to the intersection point but after checking its four neighbors.

c) passing the mask 3*3 for checked the four neighbors (right, left, top and bottom) of candidate intersection point. This mask contain only the position of the visited pixel, i.e. (contain only the position of the right point, left point, top point and bottom point). If obtained on all this four neighbors then the candidate intersection point is assigned as real intersection point of overlapped chromosomes. figure 9 is explain the four neighbors around of the intersection point and show figure 10 is present identify the intersection point.
Finally, Localize the ROI according to the intersection point, where has being draw circle on this point. Its diameter is the size of the image divided by four. Then has being draw square on this region.
6.4 Stage 4: Extract All Possible Cut Points.

To extract possible cut points will need initially, to extract the contour from binary overlapped chromosome using canny edge detection, show the figure 11.

A contour is the boundary of image i.e. is the edge detection of the image. Canny operator is used for this purpose. The Canny edge detector is an edge detection operator that uses a multi-stage algorithm to detect a wide range of edges in images. Through extraction of the contour of the overlapped chromosome, will notice that these boundaries are indirect and contain on the small a zigzag lines and straight lines and because the four intersection points are located in the very small zigzag lines, so will need to extract the zigzag lines( possible cut points) only except the straight lines within the ROI. The meaning of the straight and zigzag lines are referred in the figure 12.
Figure 12. Explain the straight and zigzag on the boundary of overlapped chromosomes.

To order computed the possible cut points, the ROI will be pass to the contour of the binary overlapping chromosome image. This operation was performed by passing the mask with size 7*7 on the contour image within the ROI only, this mask contain on the fixed positions show figure 13.

![Positions of the mask.](image)

| -3, -3 | -3, -2 | -3, -1 | -3, 0 | -3, 1 | -3, 2 | -3, 3 |
|-------|-------|-------|-------|-------|-------|-------|
| -2, -3 | -2, -2 | -2, -1 | -2, 0 | -2, 1 | -2, 2 | -2, 3 |
| -1, -3 | -1, -2 | -1, -1 | -1, 0 | -1, 1 | -1, 2 | -1, 3 |
| 0, -3  | 0, -2  | 0, -1  | 0, 0  | 0, 1  | 0, 2  | 0, 3  |
| 1, -3  | 1, -2  | 1, -1  | 1, 0  | 1, 1  | 1, 2  | 1, 3  |
| 2, -3  | 2, -2  | 2, -1  | 2, 0  | 2, 1  | 2, 2  | 2, 3  |
| 3, -3  | 3, -2  | 3, -1  | 3, 0  | 3, 1  | 3, 2  | 3, 3  |

Figure 13. Positions of the mask.

initially, the visited point will be checked whether this point is a white pixel or not. When the condition is met, will be put the mask with size 7*7 on this position where this visited point is placed in the middle of the mask and the points around it are neighboring to that point. After that computed the difference in positions between the current point (visited point) and the neighbors it, this performed by implementation an equation (1) and equation (2). When this a difference is small (less than the number 2) you can say about the visited point is one of the possible cut point, whereas if this difference is large (more than the number 2) you can say about the visited point is not one of the possible cut point. The figure (14 a, b, c, d) is show the example of this stage.

\[
| \text{count1} + \text{visited point}[x] - \text{previous point}[x] | > | \text{count 1}| \\
\]

\[
\text{count1} = \text{count1} + \text{visited point}[x] - \text{previous point}[x]
\]

\[
| \text{count2} + \text{visited point}[y] - \text{previous point}[y] | > | \text{count 2}| \\
\]

\[
\text{count2} = \text{count2} + \text{visited point}[y] - \text{previous point}[y]
\]
a) the contour image with ROI

b) the array of the contour image, N is number of rows and M is the number of columns and The current pixel is marked with position (0,0).

c) Has been put the current pixel on the mask in a position (0,0), and the other pixels are neighbors for that pixel.

d) Applying the equations on this pixels that in an above mask with considering the positions of this pixel from the original mask in a figure (8).

Initially, count 1 and count 2 = 0 for x, y respectively

For X

i- \[ |0 + (0-0)| = 0 \]

ii- \[ |0 + (0-0)| = 0 \]

iii- \[ |0 + (0-0)| = 0 \]

iv- \[ |0 + (0-0)| = 0 \]
Count 1 = 0
   For y
   \( v- \mid 0+(-1) \mid = 1 \)
   \( vi- \mid 1+(-2) \mid = 3 \)
   \( vii- \mid 3+0+1 \mid = 4 \)
   \( viii- \mid 4+0+2 \mid = 6 \)
   Count 2 = 6
   Finally, count 1 - count 2
   Difference = |(0 - 6)| = 6.

Figure 14. Show the result of this stage.

Figure 14. All possible cut points marked with red color within ROI

6.5 Stage 5:- Identified The Four Cut Points.

This stage will implementation by helping an intersection point and all possible cut points. But initially, has been perform overlay between the contour image with all possible cut points with ROI and thinning image with intersection point. Then computed the distance between intersection point and each possible cut point using the Euclidean distance as equation (3). Finally, only four minimal distances are preserved, considering this four points are located in the four directions (top right, top left, bottom right, and bottom left) ration to the intersection point. Show the figure 16.

\[
D(p,q) = \sqrt{(p.x - q.x)^2 + (p.y - q.y)^2} \quad \ldots \ldots \quad 3
\]

Where \( p(x,y) \) is the intersection point and \( q(x,y) \) is the possible cut point.
6.6 **Stage 6:- Draw Lines Between The Four Cut Points.**

In this stage will draw the two vertical lines and two horizontal lines in order to extract the common area of the overlapping chromosomes. Show the figure 1.

![Figure 1](image1)

**Figure 15. c The four cut points**

6.7 **Stage 7:- distinguish between two chromosomes.**

Every chromosome has a particular sequence of higher and lower intensity values, indicating the presence of light and dark bands on it. These bands, present on the body of chromosomes. This orientation of the bands, with respect to the axis of the chromosome, is always retained irrespective of any shape variability or any nature of the overlapping chromosome. This intensity values of the overlapping chromosomes will helping in distinguish between chromosomes. If a and b are the two overlapping chromosomes, such that b overlaps on a, then b is the topmost chromosome. The bands present on the overlapped region are vertically to the axis of the chromosome b, show figure 16.

![Figure 16](image2)

**Figure 16. Draw rectangle on the overlapped region**
Figure 17. a two overlapping chromosomes with indicating overlapped region and desired cut points, b

The overlapped region recovered by joining the identified cut-points using the banding information in that region. The conclusion from above text is the topmost chromosome having vertical banding information to the axis of the chromosome. For that, it is necessary to observe a proper direction (scan lines that lying behind the overlapped region of each overlapped chromosome in every direction) along the axis of the chromosome. The variations in the intensity values is examined along four scan lines in the horizontal and vertical direction. Show figure 18.

Figure 18. Overlapping image with four direction, 1 and 2 are vertical lines, 3 and 4 are horizontal line
In addition, usually when an object falls on the other object, the top-most object will generate a shadow on the down-most object. From this will note that there are two parallel lines of the four lines of the overlapping area has less intensity values compared with the other two lines, show figure 19.

![Figure 19](image1.png)

**Figure 19.** a The overlapping chromosomes, b explain the line 4 carries darken color compares with others lines.

Storing the intensity values along every scan line in each direction in a vector. After that, extracted minimum intensity value from this vector. Thus, this value is belong to the horizontal line (i.e. belong to the down-most chromosome), after that taken the parallel line. When the parallel lines pair were obtained will colored this lines in the black color for passing this overlapped chromosome to the segmentation process. thus the overlapped chromosome it was separated Show figure (20).

![Figure 20](image2.png)

**Figure 20.** Results of the overlapping chromosomes In V/H
6.8 Separation of Touching Chromosome

The process of separating the touching chromosomes is similar to the process of separating the overlapping chromosomes, but there is a difference between the shapes of the chromosomes overlapping from the touching chromosomes, where in the first type the chromosomes appear in one shape as shown in figure (5 a), but in the second type they appear in two different shapes (1- the chromosomes appear touched from one side of the other chromosome in a parallel form 2- the chromosomes appear touched from the beginning or end of the other chromosome in Perpendicular form), as shown in Figure 22.

Accordingly, there will be a difference in the way to find the intersection point for the overlapping chromosomes from the touched chromosomes, because in the types of the touched chromosomes there is clear not clear intersection point can be extracted from thinning of the touching chromosomes, as shown in Figure 23.
Figure 23. a The different of touching chromosomes images, b is the thinning of images a.

The separation of touching chromosomes was worked by following the algorithm in figure 24.
In the algorithm of separation touching chromosomes, the intersection point is determined in two ways:

- Either, after the thinning of touched chromosome have been extracted, the presence of the intersection point of has been examined in the same method as for identification the intersection point of the overlapping chromosomes.

- Or pass the thinning and the contour of the touched chromosome together and store the data (values) in one picture as in the figure (25). Then the mask 9*9 is passed to the image and compute the neighbors for visited pixel in the mask, and find the maximum number of neighbors of current pixel. Thus, has been assigned this the pixel to the intersection point of touching chromosome.
After that, Specified ROI and extract all possible cut points as the methods were used in the overlapping chromosome. After extracted the possible cut points, The same procedure in the fifth step of the overlapped chromosomes is done in the touching chromosome. But the difference is, in the number of the selected cut points, in the overlapping chromosomes Four cut points were selected while in the touching chromosomes will be selected only two cut points. with consideration is this points are located on the two sides of the intersection point, show figure 26.

Then draw line between this cut points, show figure 27.a. Then colored this line in the black color for passing this touching chromosome to the segmentation process, thus the touching chromosome it was separated Show figure 27.
7. Conclusion
Analysis of karyotype process is a familiar process in cytogenetics to assess the achievable presence of genetics defects. That an automatic analysis would greatly help the expert human in its routine work that performing in labs. But still automatic segmentation and full disentangling of chromosomes are open problems. In this thesis has been focus on the basic issue, which is separation touching and overlapping. The problem of separation touching and overlapped chromosome was solved using the
convulsion mask and with a help the morphological thinning and contour. The separation of touching and overlapped chromosome is according on finding cut points. The thinning of the image is obtained which helps to identification the ROI. The contour of the image is obtained which helps to explain the entire shape of the image and find out the cut points for separation between chromosomes.

8. Reference

[1] Minaee, Shervin, Mehran Fotouhi, and Babak Hossein Khalaj. 2014. A geometric approach to fully automatic chromosome segmentation. IEEE Signal Processing in Medicine and Biology Symposium (SPMB). IEEE.

[2] Madian, III Nirmala. 2012. AUTOMATED IDENTIFICATION TO THE CENTROMERE POSITION AND THE CENTROMERE INDEX (CI) OF HUMAN CHROMOSOME IN G-BANDED IMAGES, ISSN.

[3] Yan, Wenzhong, and Lei Bai. 2013. Algorithms for chromosome classification. Engineering 5:10: 400.

[4] Moradi, Mehdi, Seyed Kamaledin Setarehdan, and S. R. Ghaffari. 2003. Automatic locating the centromere on human chromosome pictures. 16th IEEE Symposium Computer-Based Medical Systems. Proceedings. IEEE.

[5] Popescu, Mihail, et al. 1999. Automatic karyotyping of metaphase cells with overlapping chromosomes. Computers in biology and medicine 29.1: 61-82.

[6] Arora, Tanvi, and Renu Dhir. 2016. A review of metaphase chromosome image selection techniques for automatic karyotype generation. Medical & biological engineering & computing 54.8: 1147-1157.

[7] Madian, Nirmala, and K. B. Jayanthi. 2014. Analysis of human chromosome classification using centromere position. Measurement 47: 287-295.

[8] Goienetxea, Izaro, et al. 2012. Image analysis pipeline for automatic karyotyping.” International Conference on Hybrid Artificial Intelligence Systems. Springer, Berlin, Heidelberg.

[9] Vijayan, Vinya, R. S. Remya, and K. Sabeena. 2015. Survey on chromosome image analysis for abnormality detection in leukemias. IJRET: International Journal of Research in Engineering and Technology 4, 664-669.

[10] Nair, Revathy M., R. S. Remya, and K. Sabeena. 2015. Karyotyping techniques of chromosomes: a survey." Int J Comput Trends Technol 22.1.

[11] Somasundaram, Devaraj, and VR Vijay Kumar. 2014. Separation of overlapped chromosomes and pairing of similar chromosomes for karyotyping analysis. Measurement 48, 274-281

[12] Hu, R. Lily, et al. 2017. Image segmentation to distinguish between overlapping human chromosomes. arXiv preprint arXiv:1712.07639.

[13] Somasundaram, D., et al. 2014. G-band chromosome segmentation, overlapped chromosome separation and visible band calculation. International Journal of Human Genetics 14.2: 73-81.

[14] Madian, Nirmala, K. B. Jayanthi, and S. Suresh. 2018. Analysis of human chromosome images: Application towards an automated chromosome classification. International Journal of Imaging Systems and Technology 28.4: 235-245.

[15] Madian, Nirmala. 2014. Segmentation of Overlapped and Touching Human Chromosome images.

[16] Munot, Mousami V., et al. 2013. Automated detection of cut-points for disentangling overlapping chromosomes. 2013 IEEE Point-of-Care Healthcare Technologies (PHT). IEEE.

[17] M.W.Azani , M.M.A.K.Mohamed. 2018. A Review of Histogram Equalization Techniques in Image Enhancement Application. J Physics, Vol.1019.

[18] Arora, Tanvi and R. Dhir. 2019. A novel approach for segmentation of human metaphase chromosome images using region based active contours. Int. Arab J. Inf. Technol., pp. 132-137.

[19] L.HE,Y.CHAO,X. ZHAO,B. YAO,H. KASUYA and A.OHTA. 2017. An Algorithm for Calculating Objects’ Shape Features in Binary Images. DEStech. Comput. Scien&Eng.aiea.
[20] Kamdi, Shilpa and R.K Krishna. 2012. Image Segmentation and Region Growing Algorithm. *Int. J. Comput. Technol.*

[21] https://users.cs.cf.ac.uk/Dave.Marshall/Vision_lecture/node35.html#figureregion_growing.

[22] A. Subasinghe, J. Samarabandu, Y. Li, R. Wilkins, F. Flegal, J.H. Knoll and P.K. Rogan. 2016. Centromere detection of human metaphase chromosome images using a candidate based method [version 1; peer review: 2 approved with reservations]. Jul.