

Automatic Segmentation of Overlapped Images

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Abstract: Chromosomes are essential genomic information carriers. The identification of chromosome abnormalities is an essential part of diagnosis and treatment of genetic disorders such as chromosomal syndromes and many types of cancer. There are different types of chromosome aberration like mapping of the common duplications, deletions, and translocations. One of the most considerations in the field of chromosome analysis is the segmentation. Currently available cytogenetic imaging software is designed to classify only normal chromosomes [9]. The automation of chromosome analysis is involving segmentation of chromosomes and classification into 24 groups [8]. The segmentation and overlap of chromosomes are a major step toward the realization of homolog classification. Resolving chromosome overlaps is an unsolved problem in automated chromosome analysis and the analysis is complicated by the occurrence of clusters of overlapped chromosomes. Current systems for automatic chromosome classification are mostly interactive and require human intervention for correct separation between touching and overlapping chromosomes, and so are unable to segregate overlapping chromosomes. In this paper, propose an automatic procedure to obtain the separated chromosomes. The overlapped chromosomes are segmented by found the interesting (concave and convex) points with the help of chain code algorithm and the interesting points [1] are located on contour of the image and then the curvature function is found to find out concave points and the possible separation lines are plotted by using all concave points and finally construct hypotheses for possible separation lines between concave points. The segmentation is carried out by means of a curvature function scheme, which proved to be successful.

Index Terms: Diagnosis, genetic disorders, automatic chromosome classification, interesting points, hypothesis.

I. INTRODUCTION



Fig 1: Touching and overlap chromosomes

CHROMOSOMES are microscopic structures containing all of an individual's genetic information. The chromosomes are composed of DNA and various proteins. The DNA contains the actual genetic code of an individual, and the proteins protect the DNA and allow the DNA to duplicate properly when the cell divides. Chromosome analysis is performed on dividing cells in their metaphase stage. In that stage, the chromosomes may be stained in a way that creates a typical band-pattern on them. A chromosome in the metaphase stage is constructed from two identical arms (chromatids), which are joined together on a common point (centromere). The centromere actually divides each chromatid into two arms. Chromosomes are essential genomic information carriers. The chromosomes formed as 22 pairs of autosomes (1–22) and two sex chromosomes (X and Y). Thus, there are 24 classes of chromosomes.

A small proportion of the population has cells each one of which has fewer or more than 46 chromosomes, implying a substantial deficit or excess of genetic material. Others have one or more structurally abnormal chromosomes, in which a section of a chromosome has moved from its usual to another position. Chromosome analysis is performed on dividing cells in their metaphase stage [9]. The metaphase stage images are shown in fig. 1. The chromosomes in a metaphase image may be bent, their arms may be joined (along points other than the centromere), their bands may be spread, and they may touch or partially overlap each other.

Thus, all these problems should be taken into consideration when processing the metaphase image. Current systems for automatic chromosome classification are mostly interactive and require human intervention for correct separation between touching and overlapping chromosomes. The separation is performed by posing a series of hypotheses that fit certain constraints [13], [17].

II. RELATED WORKS

1) Segmentation methods try to separate between touching chromosomes by classifying the chromosome pixels and the background pixels into two different segments. Since segmentation methods are general methods that do not depend on the shape of objects, their link to the specific problem of chromosome separation is weak, and, therefore, they tend to fail in cases of incomplete information. A case of incomplete information occurs; there is no separating path between chromosomes [5], [7].

2) Heuristic search edge-linking methods try to separate between touching chromosomes by searching for a minimal-cost connected path that separates between the chromosomes. The link lost connection of such methods to the specific problem of chromosome separation leads to inferior results in cases of incomplete information, such as in the case where a separating path does not exist [19].

3) The method for separating the touching chromosomes by using shape decomposition based on fuzzy subset theory [16] is reported as giving inferior results relative to other general methods. An even greater disadvantage of this method is that it also yields erroneous decompositions of single chromosomes in cases of bent chromosomes.

III. PRELIMINARY PROCESSING

The preliminary processing of the metaphase image is performed by increasing the effective sampling resolution [14] may be achieved by sampling each metaphase image in some overlapping parts. Fig. 2 is the sample overlapped and touching image.



Fig 2: Sample overlapping and Sample touching images

A. Obtain the Chromosome Contours

The relevant information of a shape is contained in its contour, for separation purposes, only the shape contours are required. The use of shape contours actually represents a successful information compaction that reduces the amount of processed data and stresses the main features of the objects. Chromosome contour determined by using a common contour-following method to track the contours of the connected segments by row and columns [2]. Fig. 3 shows the binary approximation of the overlapped image and fig.4 shows the contour of the overlapped image.

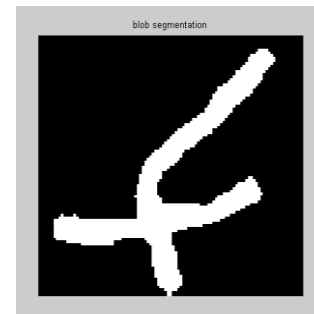


Fig 3: Binary approximation of the given overlapped image

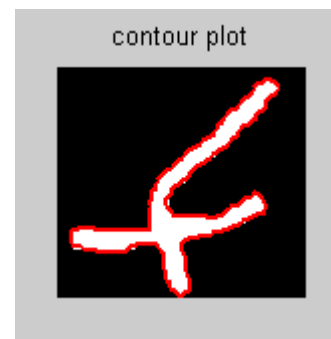


Fig 4: Contour plot of the above binary image. Contour plotted in green

B. Obtain the Discrete Curvature Function

The curvature function of a curve is defined as the rate of change of the curve slope with respect to its length. The radius of a circle is tangent to the concave side of the curve, and has the same curvature as the curve at the tangential point. The curvature function is a derivative of the contour's slope function. To reduce the computational requirements of the K-slope evaluation, an extended chain-code method is used. Using the extended chain-code, it is possible to determine the K-slope at a given pixel directly from a look-up table where the extended chain-code serves as the entry point.

This method used for detecting and locating "corners" in chain-coded curves [3]. The "corner" means that chain node with which we can associate an identifiable discontinuity in the mean curvature of the curve. The detection of a corner is a function of the magnitude of the discontinuity. The concave and convex points of the object are detected by using chain code method. Fig. 5 presents the result of interesting points detection on the curvature graph, where the interesting points are marked with triangles.

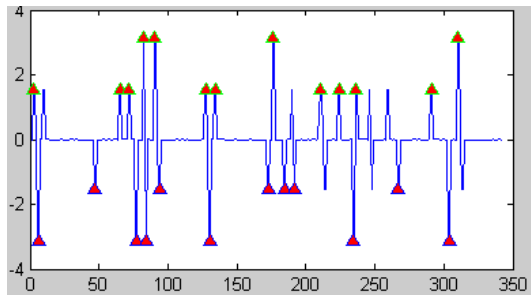


Fig 5: Interesting points on the curvature graph.

C. Obtaining the Interesting Points

The interesting points are detected as extremum points of the curvature function, or as middle points of constant curvature curve segments. The required filter should combine a low-pass filter for high frequency noise removal, and a high-pass filter for curvature peaks enhancement.

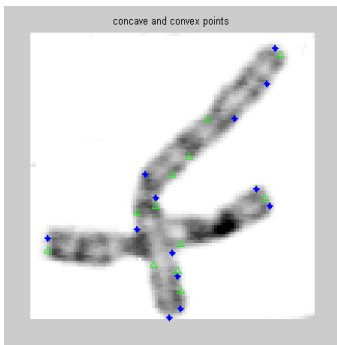


Fig 6: Interesting points on the chromosome

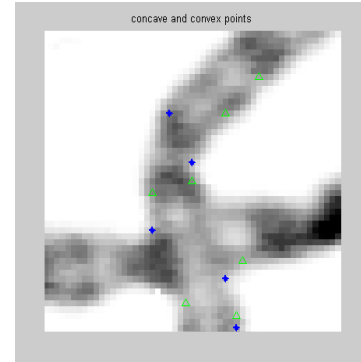


Fig 7: Extended view of the above chromosome image

Thus actually resulting in a band-pass filter. The filtered curvature function is segmented, and extremum points are detected within each segment. From that list, only concave points with a curvature measure above a certain threshold are selected. The interesting points (concave and convex points) are located on the chromosomes. Fig. 6 shows the location of interesting points on the chromosome and fig. 7 shows the extended view of fig. 6.

IV. CHROMOSOME SEPARATION

Each chromosome as a separate subset of pixels, it is possible to view the chromosome separation, the minset is obtained by taking the intersection between some subsets and the complement of the other subsets. Each minset of the chromosomes represents either a part unique to one chromosome or a part common to more than one chromosome. The minset normal form representation of the chromosomes can be constructed by a union of such parts. The required partition is obtained by splitting the object along lines that connect high concave points.

For the determination of separation lines [4], all the possible pairs of high concave points are considered. The separation lines are obtained by taking all the possible combinations of two points.

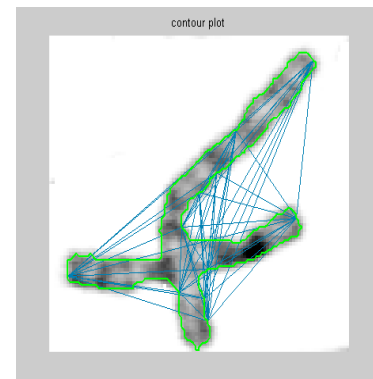


Fig 8: Possible separation lines

A. Hypotheses for Overlapping Objects

The constraint requires that the spatial distance between the vertices of a separation line of touching objects [20] should be small relative to the distance between the vertices along the shape's contour.

To construct a hypothesis for the separation of overlapping objects, an overlap minset should be located. The distance between the separation lines should be above a given threshold. Fig. 8 shows the possible separation lines between minsets.

B. Hypotheses verification

The verification of hypotheses is based on an evaluation of the fit of the obtained parts to the prototype shapes of the chromosomes [10]. In general, the shape of a chromosome is not fixed. A chromosome may have various heights and widths.

The evaluation of the fit of a chromosome to its prototype shape is done by fitting a bounding polygon to the chromosome [10], [11]. The bounding polygon is constructed by locating the shape's minimal bounding box, contracting it in the middle, and rounding its corners. In order to retain the uniqueness of the bounding polygon as a chromosome prototype, constraints are posed on possible locations of its vertices. The minimal bounding box of a shape is found by computing the principal component transformation of that shape. Fig. 9 shows the overlap region, fig. 10 shows the first chromosome segmentation from other chromosome and fig. 11 shows the second chromosome segmentation from other chromosome.

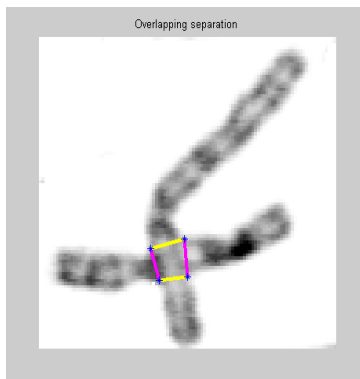


Fig 9: Region of overlapping in the chromosome

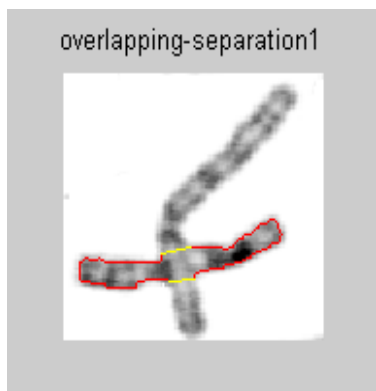


Fig 10: Separation of first Chromosome from second chromosome

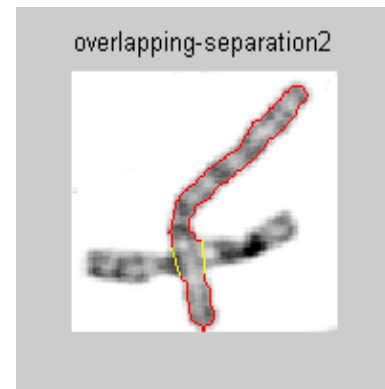


Fig 11: Separation of second Chromosome from first chromosome

V. FUTURE WORK

In the case where there are only touching objects, the problem of finding a separation hypothesis is reduced to finding the minset partition. After finding that partition, each object is composed of one minset and therefore, there is no need to find unions of minsets. Since the number of possible partitions is rather large, it is reduced by introducing an additional constraint on the possible pairs.

The constraint requires that the spatial distance between the vertices of a separation line of touching objects should be small relative to the distance between the vertices along the shape's contour.

The reduction in the number of possible partitions by using the constraint of nearly parallel separation lines. An additional reduction of the number of possible partitions is obtained by introducing a constraint on possible combinations of pairs and so possible partitions will be only part of the sets.

For example Fig. 12 shows touching region and fig. 13 and fig. 14 shows the disentangling of touching chromosomes. The experiments are going in the separation of touching chromosome with out any loss in the information [12].

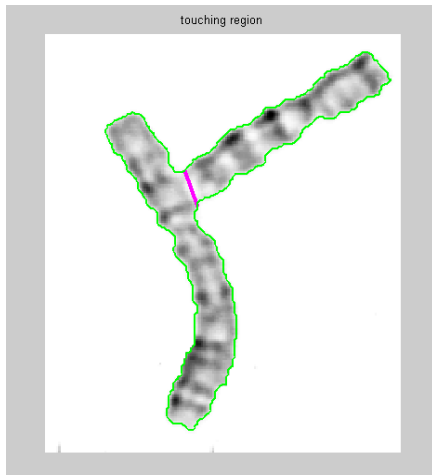
A .Sample disentangling Images

Fig 12: The region of touching chromosomes

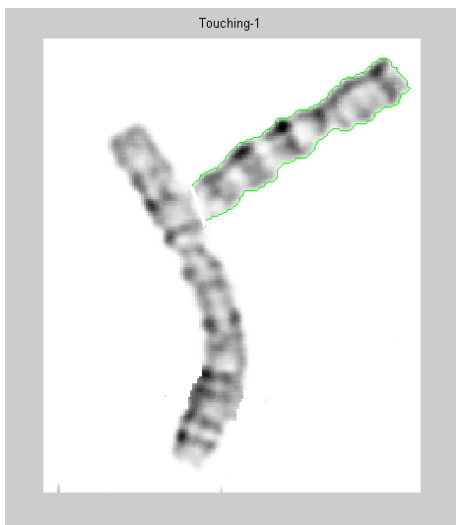


Fig 13: disentangling of first chromosome from second chromosome

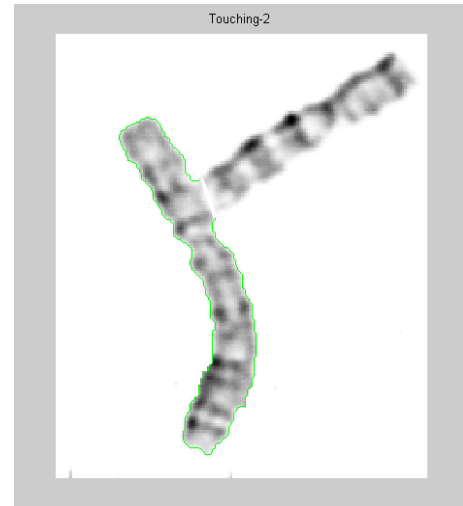


Fig 14: Disentangling of second chromosome from first chromosome

V. CONCLUSION

Karyotype analysis is a widespread procedure in cytogenetics to assess the possible presence of genetics defects. The procedure is lengthy and repetitive, so that an automatic analysis would greatly help the cytogeneticist routine work [6]. Still, automatic segmentation and full disentangling of chromosomes are open issues.

The problem of extracting individual objects from overlapping configurations is one that occurs in a number of applications in machine vision [18]. It is a difficult problem in general and can be thought of in two parts: 1) identifying overlapping objects in the first place and 2) identifying the individual components. In the case of chromosomes, it is usually fairly easy to identify an overlap on straightforward shape criteria.

This proposed method for Automatic Chromosome segmentation of touching and overlapping images are more accurate than other previous methods and this method of segmentation is applicable for large number of bents present in the chromosome structures and more number of clusters [15] by the location of each separation point is optimized, yielding the minimal possible distance between the smoothed approximation and the original curve.

The experiment proved that this algorithm can segment the overlapping chromosomes successfully.

REFERENCES

- [1] G. Agam and I. Dinstein, "Geometric separation of partially overlapping non-rigid objects applied to automatic chromosome segmentation", *IEEE Trans. Pattern Anal. Mach. Intell.*, vol. 19, no. 11, pp. 1212–1222, Nov. 1997.
- [2] E. Grisan, A. Pesce, A. Giani, M. Foracchia, and A. Ruggeri., "A new tracking system for the robust extraction of retinal vessel structure," in *Proc. 26th Annu. Int. Conf. IEEE-EMBS*. New York: IEEE Press, 2004, pp. 1620–1623.

- [3] D.L.Milgram and A.Rosenfeld, "A Corner-Finding Algorithm for Chain-Coded Curves", IEEE Trans. Pattern, 2000.
- [4] X. Bai, C. Sun, F. Zhou, Touching cells splitting by using concave points and ellipse fitting, Proceedings of Digital Image Computing: Techniques and Applications, Canberra, Australia (2008) 271–278.
- [5] K.Z. Mao, P. Zhao, P. Tan, Supervised learning-based cell image segmentation for P53 immunohistochemistry, IEEE Transactions on Biomedical Engineering 53 (6) (2006) 1153–1163.
- [6] C. Urdiales García, A. Bandera Rubio, F. Arrebola Pérez, and F. Sandoval Hernández, "A curvature-based multiresolution automatic karyotyping system," Mach. Vis. Appl., vol. 14, pp. 145–156, 2003.
- [7] M. Moradi and S. K. Staredhan, "New features for automatic classification of human chromosomes: A feasibility study," Pattern Recognit. Lett., vol. 27, pp. 19–28, 2006.
- [8] A.Carothers and J. Piper, "Computer-Aided Classification of Human Chromosomes: A Review," Statistics and Computing, vol. 4, no. 3, pp. 161-171, 1994.
- [9] M. Thompson, R. McInnes, and H.Willard, Genetics in Medicine. ON, Canada: Saunders, 1991.
- [10] Kolesnikov, A., Fränti, P.: A fast near-optimal algorithm for approximation of polygonal curves. Proc. Int. Conf. Pattern Recognition-ICPR'02. 4 (2002) 335-338.
- [11] Chen, D.Z., Daescu, O.: Space efficient algorithm for polygonal curves in two dimensional space, Proc. 4th Int. Conf. Computing and Combinatorics. (1998) 55-64.
- [12] G. C. Charters and J. Graham, "Disentangling chromosome overlaps by combining trainable shape models with classification evidence," IEEE Transactions on signal processing, vol. 50, pp. 2080–2085, August 2002.
- [13] P. Mousavi, R. K.Ward, and P. M. Lansdorp, "Feature analysis and classification of chromosome 16 homologs using fluorescence microscopy image," IEEE Can. J. Elect. Comput. Eng., vol. 23, no. 4, pp. 95–98, 1999.
- [14] K. Saracoglu, J. Brown, L. Kearney, S. Uhrig, J. Azofeifa, C. Fauth, M. Speicher, and R. Eils, "New concepts to improve resolution and sensitivity of molecular cytogenetic diagnostics by multicolor fluorescence in situ hybridization," Cytometry, vol. 44, no. 1, pp. 7 May 2001.
- [15] W. C. Schwartzkopf, A. C. Bovik, and B. L. Evans, "Maximum-likelihood techniques for joint segmentation-classification of multispectral chromosome images," IEEE Trans. Med. Imag., vol. 24, no. 12, pp. 1593–1610, Dec. 2005.
- [16] T. Law, K. Yamada, D. Shibata, T. Nakamura, L. He, and H. Itoh, "Edge extraction using fuzzy reasoning," in Soft Computing for Image Processing, S. K. Pal, Ed. New York: Physica-Verlag, 2000.
- [17] M. Moradi and S. K. Staredhan, "New features for automatic classification of human chromosomes: A feasibility study," Pattern Recognit. Lett., vol. 27, pp. 19–28, 2006.
- [18] M. Sezgin and B. Sankur, "Survey over image thresholding techniques and quantitative performance evaluation," J. Electron. Imag., vol. 13, pp. 146–168, 2004. T. Ried, "Cytogenetics—In color and digitized," New Eng. J. Med., vol. 350, no. 16, pp. 1597–1600, Apr. 2004.
- [19] Carsten Garnica, Frank Boochs, Marek Twardochlib, "A new approach to edge-preserving smoothing for edge extraction and Image segmentation", IAPRS, Vol. XXXIII, Amsterdam, 2000.
- [20] Xiangzhi Baia,, ChangmingSun, FugenZhou, "Splitting touching cells based on concave points and ellipse fitting", Pattern Recognition 42 , 2009.



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