# AUTOMATIC DETECTION OF PALE PATH AND OVERLAPS IN CHROMOSOME IMAGES USING ADAPTIVE SEARCH TECHNIQUE AND RE-THRESHOLDING

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Abstract: Detection and separation of overlapping and touching chromosomes is a critical issue in image analysis applications for cytogenetics where accurately segmented chromosomes are essential. We present a novel method of automatic pale path detection for all types of stained chromosome images. Optimum number of cut-points for each cluster of touching or overlapping chromosomes is obtained and analysed sequentially for the pale paths. A self-adaptive search window searches for the minimum grayscale intensity beginning from the vicinity of a cut-point and propagates gradually till the end of pale path. Efficient image and area thresholding restricts the faulty detection of touch or overlap in a chromosome cluster.

## 1 INTRODUCTION

Images of human metaphase cells show individual chromosomes as discrete objects, each of which can adopt varied and inconsistent morphologies. For cytogenetic analysis, it is important that the chromosomes in an image be analyzed individually in order to detect structural abnormalities responsible for congenital diseases or cancer. During preparation, chromosomes may overlap or touch one another. The degree to which they coincide with one another may significantly vary from cell to cell. The process of segmenting images into individual chromosomes involves defining the boundaries of chromosomes. This procedure needs to be both accurate and comprehensive in order to detect chromosome abnormalities.

In order to analyse the structure of individual chromosomes, a digital cell image has to be segmented accurately. The segmentation process can be challenging mainly due to intensity dispersions and overlapping between chromosomal bodies. Detecting and separating touching and overlapping chromosomes in a digital cell image will improve segmentation accuracy significantly.

Manual detection and separation of occluded chromosomes in digital images can be very time consuming. Early work in automating this process included applying a variety of segmentation methods like heuristic edge-linking, region growing, fuzzy-logic, etc. A rule based chromosome segmentation procedure (Liang, 1994) explores the possibility of using a rule based classification and segmentation parameter set for each cell. In the knowledge-based chromosome contour searching method (Agam and Dinstein, 1997) an edge-preserving smoothing nonlinear filter is applied to remove random noise while preserving the edges of chromosomes. A classification driven partially occluded object segmentation (CPOOS) method was proposed to resolve partial occlusion in chromosome images (Lerner, Guterman, and Dinstein, 1998). Minimum entropy segmentation algorithm was also investigated to decompose or separate groups of chromosomes that touch and overlap each other (Schwartzkopf, 2001). Automatic segmentation of metaphase cells was also discussed based on global context and variant analysis (Ritter and Gao, 2008). Watershed algorithm also finds its utilisation in segmentation of narrow-touching chromosomes (Ming and Tian, 2010). Most of the procedures

discussed above cater only to a particular type of banded chromosomes. They fail in analysing any chromosome images that are stained in a different manner than what the procedure demands.

In this paper, we propose an algorithm that automatically separates the touching chromosomes and detects overlapping chromosomes by optimum thresholding and self-adaptive search window for pale path detection. Unlike other methods, this algorithm is applicable for all types of stained chromosome images.

The rest of the paper has been organized as follows. Section 2.1 provides an insight into the classification of chromosome images into two basic categories. Section 2.2 introduces the proposed method of obtaining high concavity points using moving average filter while section 2.3 presents the proposed method of local thresholding for pale path detection in case of touching chromosomes. A given area constraint is also applied to prevent segmentation of unwanted small groups of pixels from a chromosome and is discussed in section 2.4.

# 2 PROPOSED ALGORITHM

## 2.1 Image Classification

The chromosome images can be broadly classified into two groups: one with more of "cross-like" chromosomes (in which sister chromosomes have prematurely separated) and another in which the sister chromosomes have coalesced into a single, uninterrupted homologous pair.We apply an automatic classification mechanism based on counting branch-points of the binarized and thinned image. This classification plays crucial role in decision making of pale path (Section 2.3).

## 2.2 Determination of cut-points

A window size of 200 x 200 pixels is manually selected from nuclei free grayscale image containing the object of our interest as shown in Figure 1.

(a) (b)

Figure 1: (a) Binary image of the selected object. (b) The single pixel thick contour developed from the given binary image.

To determine the points of concavity in the contour, we use the exterior angle of boundary pixels (calculated between adjacent pixels). Due to the discreteness of the pixels, they belong to the set of angles {π/2,3π/4, π, 5π/4 and 3π/2}

A high concavity point in the boundary layer demands following characteristics.

- Its angle should be either 3π/4 or π/2
- The mean of the angles for a given set of neighbourhood [x-N, x+N] must be less than π

$$\left(1/2N+1\right)\sum_{i=-N}^{N} angle(x+i) < \pi \qquad (1)$$

- This mean obtained above should never exceed π for all the sets of neighbourhood less than (+N) range of that point.

$$\left(1/2K+1\right)\sum_{i=-K}^{K} angle(x+i) < \pi, \text{ for all } K < N \quad (2)$$

To obtain the mean of the angles, a moving average filter with a width of 3 is applied to the set of angles.

Moving average filter is then applied with iterating filter width initially set to 1. The iteration stops if the mean angle value exceeds pi or when the iterations reach N cycles. To meet all the 3 conditions specified above for a concave point, we define two sets of boundary pixels X and Y. Set X contains all the pixels having angles π/2 or 3π/4. Set Y comprises of pixels with angles after application of moving average filter being less than π. The intersection of Set X and Set Y gives us the desired high concavity points or cut-points.

## 2.3 Singularity/touch/overlap detection

An object is classified as a single chromosome if the number of cut-points (NCP) is less than 2 because any overlap or touch requires at least two high concavity points. If their number is more than 2, we try to find a pale path originating from each cut-points to judge whether the object contains touching chromosomes (section 2.3.1). Once the pale path succeeds in dividing the object, the sub-parts are further analysed individually by finding out their NCPs. If they exceed the value 2, we go for the search of pale path in that sub-part until there are no more pale paths possible. Each newly sub-parts formed are analysed in the same fashion till all the cut-points are analysed for pale paths.

After analysing all the high concavity points, the sub-parts are fed through morphological thinning operation and again the X or T intersections are checked. We already know whether an image constitutes mainly of homologous chromosomes in which sister-chromatid separation occurs (class A)

or does not (class B). In general, an X shaped chromosome consists of 2 T-intersections or nodes whereas a normal chromosome does not contain any of them. Therefore, a sub-part with number of cut-points greater than or equal to 2 is considered a single chromosome, if it contains 2 or more skeleton nodes for the image of class A or no node for the image of class B. Any sub-part not complying with this rule is tagged as overlap.

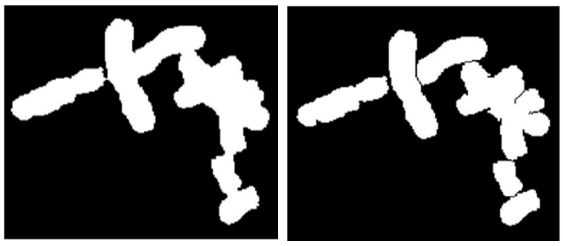
### 2.3.1 Pale path detection

We can categorize the cut-points into three different types depending on their local convergent angles: $\pi/2$, $3\pi/4$ and $\pi$. The cut-point with convergent angle $\pi$ is resultant for close vicinity cut-points reduction. All the cut-points are set to '0' in the binary image. For each of them, a 3x3 search is defined. Smallest intensity within this window is then set to 0, which is then used as the center of next iteration. The iteration terminates only when the centre reaches the boundary of object or the size of the search window is reduced to 0.

An important point to note here is that the iterative 3-pixel window search won't always provide the correct pale path. It will simply find a path from one boundary end to another irrespective of the intensity value traced. That may lead to dissection of "cross-like" chromosome which would result in false positive pale path. To overcome this limitation, we classify the object further into high intensity regions and pale regions. Only those pixels which lie in the pale regions are searched for minimum intensity in the 3-pixel window. If none of the pixels of the window lie in the pale region, then the search iteration is stopped, which indicates a "cross-shaped" chromosome. The pale paths detected for a group of 8 overlapping and touching chromosomes is shown in Figure 2.

The classification into relatively high intensity and pale regions is done by re-thresholding of the object. The Otsu method applied to the object forms a pale region that may contain the pale path as well as a lot of the unwanted surrounding area. To avoid this, we propose a novel method of classification using the object's image histogram.

It can be clearly seen that the pixels of grayscale intensity less than the Otsu's threshold plus an offset can be subdivided into two regions: relatively high intensity region comprising of chromosome bands and relatively low regions comprising of pale paths. In the envelope of the image histogram plot, we find peaks corresponding to these two regions. Our aim is to find the threshold around the minima to separate the pale path region from the unwanted dark bands. This is fulfilled by applying Intermeans Method on the pixels less than the sum value of object's Otsu's Threshold and the offset (set to 10) so that the search window pixels are formed of grayscale intensity less than the new threshold.



(a) (b)

Figure 2: (a) Binary image of a cluster of overlapping and touching chromosomes. (b) The pale paths detected for touching chromosomes.

For the case of class A type chromosomes, the Intermeans method becomes inefficient because of the large number pixels in the pale regions. To compensate for this, Intermeans method is applied only between the maxima of the two peaks of image histogram. Hence, the reduced range of intensity values increases the accuracy for this method.

The new threshold divides the object in a more constricted pale region. A pixel in a search window is applicable for search only if it lies in the pale region obtained from the final threshold. When none of the window pixels are a part of that region, the search process stops.

## 2.4 Optimum area criterion

Sometimes, a small-sized extension of the chromosome may split itself if it lies in the pale region generated. To avoid such kind of anomalies, we restrict the 'pale-path splitting' by an area threshold. Only those sub-parts with a size more than the area threshold can split to form a separate chromosome. The area threshold is decided by computing the mean and standard deviation (SD) of the area of all the objects of binary image. Objects with very large area are excluded, as they have a high chance of containing an overlap. The minimum area of an object is also calculated and stored ($A_{min}$). We define an area threshold which is given by:

$$A_{th}^{i} = \text{Mean} - (i/2)*\text{SD}, \quad i \in I \qquad (3)$$

We increment 'i' starting from 1 and compute $A_{th}^{i}$ successively. At each iteration, $A_{th}^{i}$ is compared against $A_{min}$. The iteration stops when $A_{th}^{i}$ becomes less than this minimum area. The final threshold area is chosen to be $A_{th}^{i-1}$ that is, the area threshold just greater than $A_{min}$.

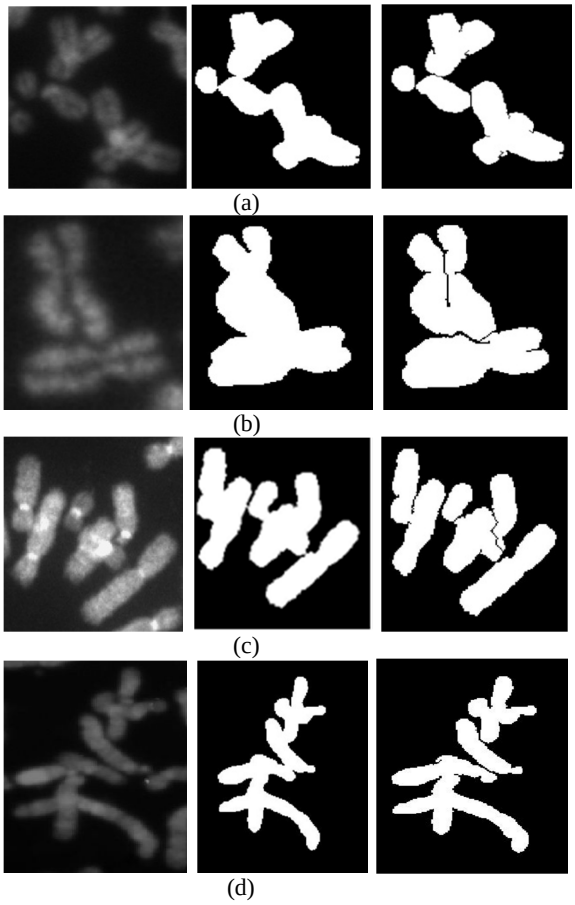


Figure 3: Pale path detection in chromosomes, left: grayscale image, centre: binary image, right: binary image with pale paths. (a) Class A, non-banded. (b) Class A, banded. (c) Class B, non-banded. (d) Class B, banded.

Hence, $A_{th}^{i-1}$ is taken as the minimum area threshold. This area thresholding ensures prevention of unnecessary splitting of very small pixel groups in the pale region of object.

# 3 RESULTS AND DISCUSSION

The method discussed above was tested for successful separations of touching chromosomes using a variety of chromosome images. It was tested with 'cross-like' chromosomes as well as smooth curvature chromosome images in which it succeeded in providing reliable results. Pale path, touch and overlap detection in a typical chromosome cluster of 'Class A' and 'Class B' images for both banded and non-banded chromosomes is depicted in figure 3.

The proposed does not require any prior set of knowledge, hence minimising the complexity. It also requires minimum pre-processing and post-processing techniques, hence further reducing computational burden. Optimum area and intensity thresholds restrict any plausible flaw that may arise during pale path detection.

# 4 CONCLUSIONS

This paper introduces a simple and effective method for pale path detection to separate touching chromosomes in images of metaphase cells. A low order finite impulse response moving average filter is used to evaluate potential cut- points. Efficient re-thresholding helps in discarding the unwanted search regions, especially those which produce false paths due to chromosome banding.

An analogous approach can be used for the segmentation of overlapping chromosomes so that they can also be analysed for valuable information. This would involve the length ratio of the arms of overlapping 'class A' chromosomes. Multiple overlaps could also be separated by first computing the number of overlaps and satisfying the arm length ratios accordingly.

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