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Identifying touching and overlapping chromosomes using the watershed transform and gradient paths

Petros Karvelis [a,b], Aristidis Likas [a], Dimitrios I. Fotiadis [b,*]

[a] Department of Computer Science, University of Ioannina, GR 45110 Ioannina, Greece
[b] Unit of Medical Technology and Intelligent Information Systems, Department of Materials Science and Engineering, University of Ioannina, GR 45110 Ioannina, Greece

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ABSTRACT

Automation of chromosome analysis has long been considered as a difficult task. However the advent of Multiplex Fluorescence In Situ Hybridization (M-FISH) made the analysis of chromosomes much easier. Nevertheless, the chromosomes in an M-FISH image do very often partially occlude each other; hence, their segmentation is not trivial and requires the application of a dedicated procedure. In this paper a method is presented for the segmentation of touching and overlapping groups of chromosomes in M-FISH images. Initially, the watershed transform is applied and the image is decomposed into watershed regions. Next, gradient paths starting from points of high concavity are computed for each produced region. Finally, adjacent regions are merged producing the final chromosome areas. To validate our method a benchmark database of 183 M-FISH images has been used. The proposed algorithm resulted in a 90.6% success rate for touching chromosomes and 80.4% for overlapping groups of chromosomes.

1. Introduction

Chromosomes are structures that contain the genetic information of cells. In a normal, nucleated human cell, there are 46 chromosomes represented in the clinical routine by a structure called the karyotype. The karyotype shows the complete set of chromosomes organized into 22 classes (each of which consists of a matching pair of two homologous chromosomes) and two sex chromosomes, XX in females or XY in males (Thompson et al., 1991). Producing a karyotype of a cell is of practical importance since it greatly facilitates the detection of abnormalities in the chromosome structure as shown in Fig. 1. Normally, the procedure of assigning each chromosome to a class (karyotyping) is based on the visual scanning of chromosome images by experts (biologists or cytogeneticists) (Thompson et al., 1991). This visual inspection is a time consuming and expensive process. Hence automated image chromosome analysis is still an important problem.

A technique was developed in the mid 90s to stain chromosomes with multiple colours so that each chromosome class appears with a distinct colour (Speicher et al., 1996). In this technique all chromosomes are labelled with five fluorophores. Also a DNA stain, called DAPI (4′,6-diamidino-2-phenylindole), is used to stain all the chromosomes with the same colour. The fluorophores attach to specific sequences of DNA, thus each pixel of the new multispectral image is represented as a five-dimensional vector, where each element of the vector represents the magnitude of the dye at that pixel of the image, Fig. 2. This technique not only facilitates the detection of subtle chromosomal aberrations (Veldman et al., 1997), but also makes the analysis of chromosome images easier; both for human inspection and computerized analysis. However, in practice, fluorophore absorption is not binary and there is significant overlap between each of the fluorophore absorptions along with variability in signal strength. This leads to a non-trivial classification problem, especially in the context of touching or overlapping regions (Schwartzkopf et al., 2005).

Many attempts have been made to automate parts of the chromosome M-FISH image analysis procedure (Schwartzkopf et al., 2005; Sampat et al., 2005; Wang and Castleman, 2005; Wang and Dandpat, 2006; Karvelis et al., 2008). However, chromosome images are inherent with the partial occlusion and touching of chromosomes, as shown in Fig. 3. This is one of the major factors hindering automatic analysis. Spectrum based methods use a pixel-by-pixel classifier to classify each pixel of the M-FISH image and this information may be sufficient to segment touching and overlapping chromosomes (Schwartzkopf et al., 2005). However the measured fluorescence at a pixel may be the combination of fluorescence in a neighbouring region leading many times to misclassification errors. These factors make the pixel spectral information of touching or overlapping chromosomes unreliable. Hence the spectral information alone cannot separate the touching and overlapping chromosomes efficiently.

On the other hand there is a variety of geometric separation based methods proposed in the literature for greyscale

* Corresponding author. Tel.: +30 26510 08803; fax: +30 2651007092.
E-mail address: fotiadis@cs.uoi.gr (D.I. Fotiadis).

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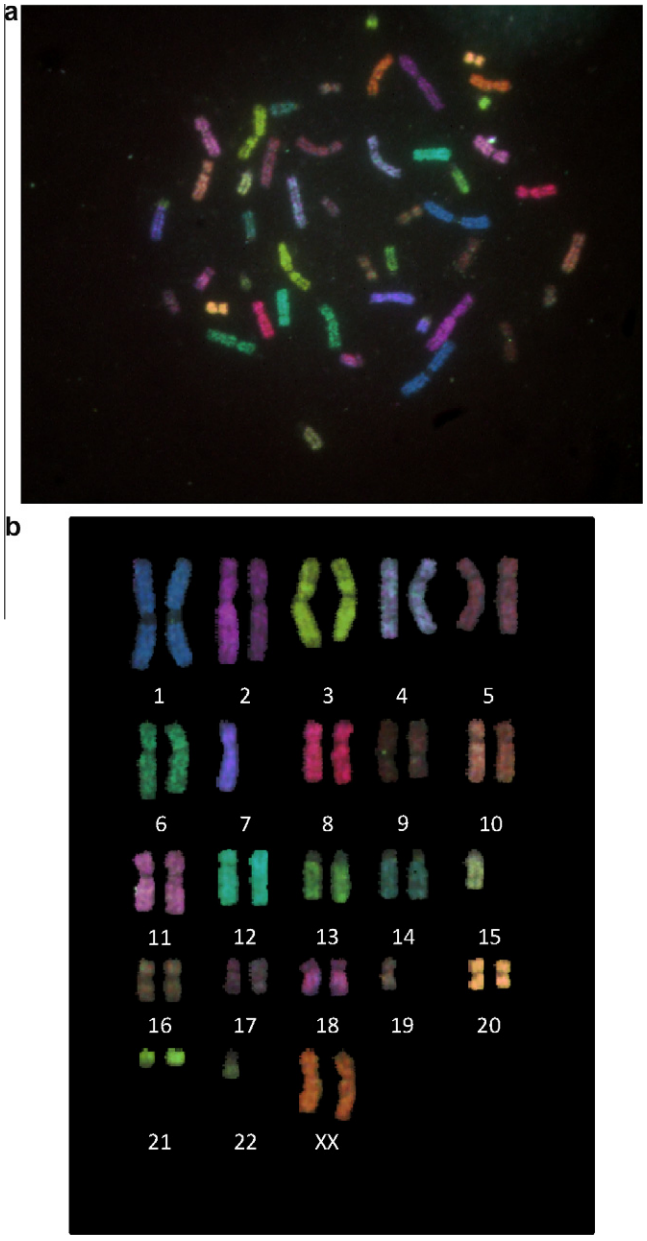

Fig. 1. (a) M-FISH chromosome image of a woman missing three chromosomes from classes 7, 15, 19, and (b) Karyotype of the M-FISH image: 43XX, -7,-15,-19.

chromosome images (Ji, 1989, 1994; Agam and Dinstein, 1997; Ritter and Schreib, 2001). The main idea of these methods is that they split the chromosome groups into segments and then they try to combine these segments into chromosomes. Valley searching techniques (Ji, 1989, 1994) attempt to find a "pale path" of grey values corresponding to a separation between touching–overlapping groups of chromosomes. Initially, all high concavity points (cut-points) are detected along the boundary of chromosomes. Next, a heuristic search is performed to detect the minimum density path between touching chromosomes. The chromosome group is split by the pale path and the segments are combined to form separate chromosomes. Agam and Dinstein (1997) used concave points to construct all the possible separation lines. In their work, they determined potential chromosomes using rectangle hypothesis testing. However this hypothesis does not always hold because of the existence of bended chromosomes that are touching or overlapping to each other and thus a straight line cannot split exactly the chromosomes.

We can conclude that when only the spectral information is used, the segmentation accuracy relies on the pixel-by-pixel classification accuracy. On the contrary, the geometry based methods assume that chromosome shape alone is sufficient for the purpose of separation. Thus both, geometry and spectral information, has to be merged in order to achieve better segmentation results for M-FISH chromosome images.

In this paper we present a novel segmentation method that tackles the problem of touching–overlapping group of chromosomes. Initially, the method uses the watershed transform to segment the DAPI image into watershed regions. The watershed transform has been widely used for the separation of touching/overlapping groups of objects from images (Beucher, 1992; Vincent and Soille, 1991; Malpica et al., 1997; Chen et al., 2003). In our case we propose the recursive application of the watershed transform to each watershed region. However there exist difficult cases of touching as also of overlapping groups of chromosomes that need separation. For this reason we use a geometry method such as the "gradient paths" to split each group of touching–overlapping chromosomes. However we do not compute the gradient paths using the intensity of pixels of the DAPI image, but we propose the computation of paths in the M-FISH image using pixels with high multichannel gradient magnitude values. This computation proves to be more efficient than the computation of the gradient path on the DAPI image since there are cases of touching or overlapping groups of chromosomes where the gradient path on the DAPI image is difficult to compute since the chromosomes are difficult to disentangle. Finally, after path computation, a region adjacency graph is computed and a region merging algorithm is used to merge all regions. In Section 2 the methods are presented describing in detail all the steps. Section 3 presents the results of the methodology. The discussion of the results is also presented in this section. Finally the conclusions of our work are presented in Section 4.

2. Method

The proposed method consists of three stages as it is shown in Fig. 4: (a) the recursive watershed transform computation, (b) the computation of each gradient path and (c) the region merging process. The first stage consists of a number of steps. The first step is the conversion of the initial DAPI chromosome image to binary. In the second step, the Euclidean distance transform of the binary image is computed. The watershed transform is applied in the next step and an initial estimation of the segmented chromosome areas is obtained. The watershed transform is further applied separately to every segmented area until no more new areas are created. The first step of the second stage is the computation of the high concavity points along the boundary of each chromosome area. Next, all gradient paths are computed and the binary chromosome area is split along the gradient path. All gradient paths are computed using the multichannel gradient magnitude. In the final stage a recursive region merging procedure is applied as follows. A region adjacency graph is computed and also each region is classified independently using a region Bayes classifier. Then we merge all neighbouring regions that share the same class. The identification of the overlapping chromosomes takes place in the final step.

2.1. Recursive watershed segmentation

In the first step, the DAPI chromosome image is converted to binary using a well known automated threshold selection process (Otsu, 1979). Using the DAPI channel an initial estimation of the regions of the M-FISH image is produced. The threshold operation at grey level l partitions the pixel values of an image into two classes