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THEORETICAL ADVANCES

Automated classification of touching or overlapping M-FISH chromosomes by region fusion and homolog pairing

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Abstract Automated separation and classification of touching or overlapping chromosomes in a metaphase image is a critical step in computer-aided chromosome analysis. The advent of the multiplex fluorescence in situ hybridization (M-FISH) technology enables multi-spectral chromosome image with rich spectral information and DAPI image with abundant texture information. This paper presents a fusion classification scheme to improve the segmentation of overlapping and touching chromosomes. First, the texture and spectral information is fused to partition the chromosome cluster into a series of homologous regions. Then a graph-theoretical classification and pairing method is proposed to resolve any remaining ambiguity of the aforementioned separation process. Experiment results demonstrate that the proposed method outperforms conventional multi-spectral classification methods in touching and overlapping chromosome separation.

Keywords Multi-spectral imaging · Chromosome image classification · Chromosome · Information fusion · Homolog pairing

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1 Introduction

Chromosomes are genetic information carriers. When chromosomes are photographed during cell division, the images of these chromosomes contain crucial information about the health status of an individual. Chromosome analysis has been an important procedure in clinical and cancer cytogenetic studies [1–3]. The classification of human chromosomes is called karyotype. Karyotyping is a useful tool to detect deviations from normal cell structure, since abnormal cells may have an excess or a deficit of chromosome. A normal human cell has 46 chromosomes consisting of 23 pairs of chromosomes, one of each pair coming from the father and the other from the mother. Of the 46 chromosomes, there are 22 homologous pairs and two sex chromosomes from the X and the Y class depending on the gender of the tested individual (two X chromosomes for a female, and one X and one Y chromosome for a male) [4, 5]. Figure 1b shows the image of a karyotype of the chromosomes in Fig. 1a. In the past, karyotyping used to be performed by trained cytogeneticists. This makes karyotyping a time-consuming and expensive operation. There are medical and economic motivations to automate the karyotype generation process; however, automated image chromosome karyotyping (or classification) is still an open topic.

Automated chromosome classification has been an active research topic in the last several decades [1, 6–9]. These methods are based on geometric (such as chromosome size, shape, morphology features) and banding information, and can work well for separated chromosomes. Chromosome images are inherent to the partial occlusion and touching of chromosomes. This is one of the major factors hindering automatic analysis. However, previous geometric and banding based classification

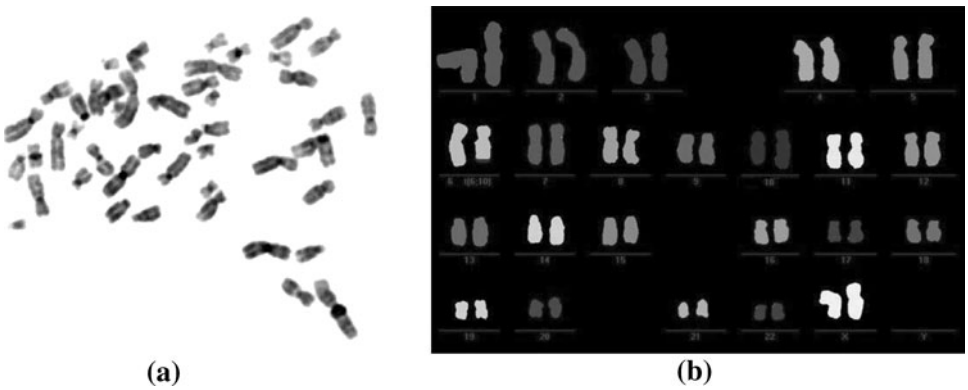

Fig. 1 **a** A metaphase cell spread; **b** a karyotype of chromosomes in (**a**)

method failed to deal with touching and overlapping. Like many pattern recognition problems, the success of automated chromosome classification depends largely on the effectiveness of the underlying feature representation scheme. With multiplex fluorescence in situ hybridization (M-FISH) technology [2–4, 10–16], chromosome analysis has become easier for visual inspection as well as computer analysis. M-FISH uses five different fluorophores that attach to specific sequences of DNA in a way that each class of chromosomes absorbs a unique combination of dyes (see Fig. 2). An extra flurophore called the DAPI (4 in 6-diamidino-2-phenylindole) is counterstained by all chromosomes. 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 [4, 17].

Based on the physical characteristics of M-FISH imaging process, and the image's feature of M-FISH, in this paper, we propose a fusion classification scheme for the automatic analysis of the M-FISH image. First, a multi-channel region fusion and classification method is proposed to divide the whole chromosome image into a series of homogeneous regions. Then these homogeneous regions are combined into potential chromosomes based on the spectral similarity.

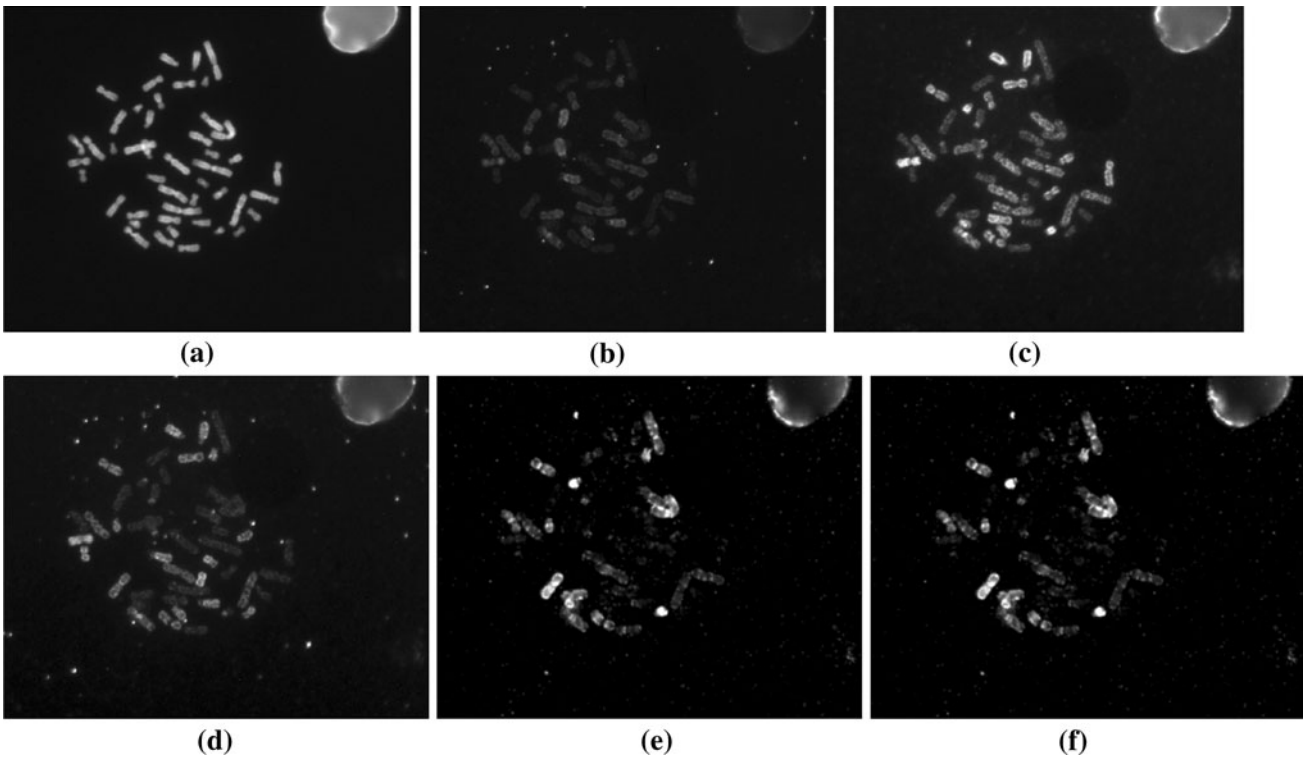

Fig. 2 An M-FISH image. Chromosomes are combinatorially labeled using five fluorophores and counterstained using DAPI. Each grayscale image corresponds to the sum of intensities of the emission wavelengths (a narrow range of wavelengths) of each fluorophore. **a** DAPI. **b** Texas Red. **c** Green. **d** S. Orange. **e** Cy 5.5. **f** Cy 5

However, there is ambiguity in choosing the optimal combination. For example, in Fig. 3a, regions A, B and D have similar spectrum, and there are six possibilities to form two chromosomes. Finally, a graph-theoretical pairing method is introduced to resolve any remaining ambiguity of the aforementioned segmentation process.

Specifically, the contributions of this paper are as follows:

1. Through combining the region-based classification and decision-level homolog pairing, a joint classification and pairing method is proposed to separate the touching/overlapping chromosomes.
2. The M-FISH image provides abundant texture and spectral information about the chromosomes, and there is complementary between them when separating the touching/overlapping chromosome. A novel overlapping/touching chromosome segmentation method is proposed based on the texture and spectral information provided by M-FISH images.
3. The classification algorithm based on region is more robust to nosy painting.

The rest of the paper is organized as follows. In Sect. 2, a brief introduction about previous chromosome classification methods is given. In Sect. 3, by jointly utilizing the spectrum and texture information, we present a multi-channel region fusion and classification method to split the chromosome clusters into a series of homologous regions. Then in Sect. 4, we present a homologous pairing method for proper region combination. In Sect. 5, experiments are carried out to validate the separation accuracy of touching and overlapping chromosome clusters in comparison with geometry-based and spectrum-based methods.

2 Related works

Numerous methods have been developed to automate the analysis process in FISH (fluorescent in situ hybridization) chromosome images [1, 5–9, 18] and M-FISH images [1, 3, 4, 10–17, 19]. For conventional FISH chromosome image, the geometry information (such as curvature, skeleton and convex hull) is utilized to separate the touching or overlapping chromosomes [1]; then, chromosomes are classified according to the banding pattern [5–7, 18]. The geometry-based method only analyzes the boundary shape of chromosome clusters. The reliability of these methods depends on two factors: splitting method and combination scheme. However, the geometric information is not reliable in many cases to segment the touching and overlapping chromosomes: for example, two chromosomes touched by their short side or long side, forming a long chromosome [1]. Spectrum-based methods assume that the spectral information is accurate enough, and the pixel classification method may be sufficient to segment touching and overlapping chromosomes. But this classification method will be dominated by noisy painting inhomogeneities [17, 20] and it fails to segment the touching and overlapping chromosomes that have similar spectral signatures [4]. All these spectrum-based methods only utilize the DAPI image to generate a mask, while ignoring the other information about chromosome contained in the DAPI image, such as texture or banding. Although texture itself is not enough for separation and classification of touching or overlapping chromosome cluster, it provides complementary data to spectral information.

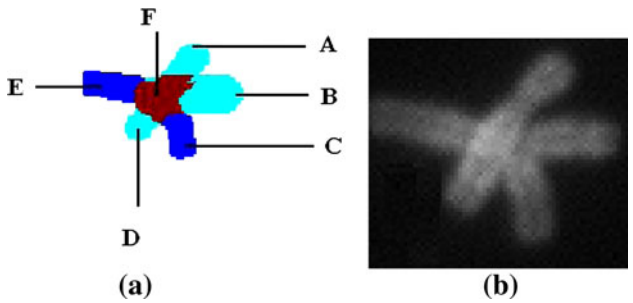

Fig. 3 Example of three overlapping chromosomes: **a** the color representation, **b** the texture representation

Table 1 presents the advantages and limitations of related studies on chromosome automatic classification, which appeared in the literature. Regardless of the information being used, the classification of touching or overlapping chromosomes can be considered as a divide-and-conquer process. This means the chromosome image is partitioned into a series of homogeneous regions where every region is purely one chromosome or part of one chromosome; then these regions are combined into corresponding chromosomes. So, the problem is how to get the homogeneous regions and how to combine these regions to form corresponding chromosomes optimally.

M-FISH image not only provides the spectral information about the chromosomes, but also the texture information of the chromosome (DAPI) image. Through combining the spectral and texture information, we can get a more homogeneous region. Homology is an important character of human chromosome; based on this information, an optimal homogeneous region combination and classification method is proposed to obtain accurate separation and classification results.

3 Multi-channel region fusion and classification

The whole M-FISH image can be separated into two groups: a five-dimensional multi-spectral image and a one-

Table 1 Automatic classification method comparison

	Method	Years	Advantages	Limitations
For FISH image	Ji [9]	1994	Fully automatic chromosome segmentation	Cannot deal with clusters of more than two chromosomes
	Biyani et al. [5]	2005	Joint classification and pairing by using the banding pattern of chromosome	Just performed on well-separated chromosomes
			Optimal classification method	
	Grisan et al. [14]	2009	Automatic separation touching/overlapping chromosome	Distinguish cluster based on the curvature and sizable bulges
				No optimal separation scheme
For M-FISH image	Sampat et al. [16]	2005	Pixel-by-pixel classification method	Does not handle touching/overlapping chromosomes
	Choi et al. [13]	2006	Region-based chromosome segmentation and classification method	Small number of testing images
				No optimal segmentation scheme
	Schwartzkopf et al. [4]	2005	Handle overlapping/touching chromosomes	Low pixel-by-pixel classification accuracy
			Use of large number and various case of M-FISH image	No optimal segmentation scheme
	Karvelis et al. [15]	2008	Region-based segmentation-classification	Does not handle touching/overlapping chromosomes
			High classification accuracy	

dimensional DAPI image. Multi-spectral image contains abundant spectral information to distinguish different chromosome classes. The DAPI image contains texture information and accurate boundary information of the chromosome that can be used to separate the chromosome from the background and distinguish chromosome regions with different textures. It can be proven from Fig. 3 that although the spectrum of region A and B are the same, their texture information is different.

For these two group images, unsupervised clustering is used, respectively, to extract the regions with homogeneous spectral information or homogeneous texture information. After homogeneous region extraction, there are two clustering maps: texture-based clustering map C_b and multi-spectral-based clustering map C_m. Here, we suppose that there are totally R different homogeneous regions in two clustering maps, and the spectral character and texture information in the same homogeneous region are similar. For a homogeneous region r, the average value of the spectrum is named as the average spectrum and it is denoted by S_r. It will be classified into 1 of 24 classes, denoted by $\Omega = \{\omega_1, \ldots, \omega_{24}\}$ (we assume that the background has been removed). The two clustering maps can be fused and classified according to the following rules:

1. If the two homogeneous regions are similar (that means these two regions have the same contour and size), these regions are named as a regular region and a posteriori probability of the region belongs to class ω_k, given the average spectrum S_r can be calculated as follows:

$$P(\omega_k|S_r) = \frac{P(S_r|\omega_k)P(\omega_k)}{\sum_{k=1}^{25} P(S_r|\omega_k)P(\omega_k)} \quad (1)$$

where $P(\omega_k)$ is a priori probability that a feature S_r belongs to class ω_k and $P(S_r|\omega_k)$ is the class conditional probability distribution function, which represents the probability distribution function for a feature vector S_r, given that S_r belongs to class ω_k.

2. If the two homogeneous regions are not similar, and the texture region is smaller than the multi-spectral region from the multi-spectral clustering map, the texture homogeneous region is named as the anomalous region. It may belong to touching/overlapping chromosomes with the same class label. The anomalous region remains and the a posteriori probability of the region belongs to the class ω_k that is calculated according to formula (1).
3. If the two homogeneous regions are not similar, and the multi-spectral region is smaller than the texture region, and suppose there are M regions, the multi-spectral region is held and the a posteriori probability of the region belongs to class ω_k that is calculated according to Eq. (1).

$$P(\omega_k|S_1, \ldots, S_M) = \frac{\prod_{j=1}^{M} P(S_j|\omega_k)P(\omega_k)}{\sum_{k=1}^{25}\prod_{j=1}^{M} P(S_j|\omega_k)P(\omega_k)}. \quad (2)$$

If $P(\omega_k|S_1,\ldots,S_M) - P(\omega_k|S_r) < \varepsilon$, then the multi-spectral region is assigned as a regular region, or else the texture region is assigned as the regular region. Where ε is the given threshold, the acquired region is named as the regular region.

By fusing the decisions made from the two information sources, the different discrimination information provided by different information sources can be effectively exploited and we get a series of regular regions and anomalous regions with temporary class label. The label is decided by maximum posteriori probability. The next step is how to combine these regions into corresponding chromosomes. To solve this problem, we use the homogeneous pairing for the globally optimal combination of segments.

4 Joint classification and pairing

During the homogeneous region merging process, we combine regions surrounded by background into corresponding chromosomes. But there is ambiguity when touching/overlapping chromosomes with similar spectrum. For these factors, only pixel-level method cannot acquire the optimal segments combination.

Here, we give two examples to show the ambiguity in the segments combination process. For example in Fig. 4b, if regions A, B, C and D have a similar spectrum, there are three possibilities [(A + E + B, C + E + D), (A + E + D, B + E + C), (A + E + C, B + E + D)] to form two chromosomes. In Fig. 4a, if regions A, B and C have different spectra, there are seven possibilities [(A + D, B + D, C + D), (A, B + D, C + D), (A + D, B, C + D), (A + D, B + D, C), (A, B, C + D), (A, B + D, C), (A + D, B, C)] to form three chromosomes. Pixel-level information alone cannot resolve these ambiguities, and there is a need to introduce high-level information.

A normal human cell contains 22 homologous pairs of chromosomes and two sex chromosomes (two X for female and one X and one Y for male). The separated chromosomes (refer to Sect. 2 for chromosomes separation) should be paired into 23 homologous pairs (for female) or 22 homologous pairs plus two independent chromosomes (for male). Based on these constraints, the segments can be combined with the guidance of homolog pairing, and it can eliminate the combination ambiguities to acquire the optimal segment combination.

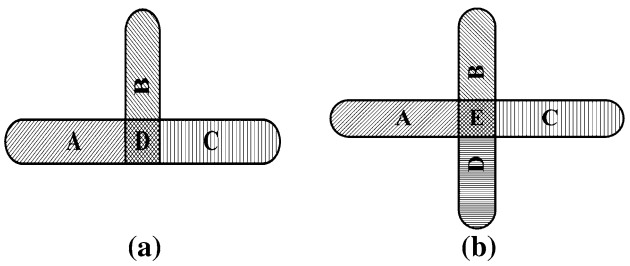

Fig. 4 An illustration of the ambiguity problems in chromosome segmentation

4.1 Homolog pairing

Any chromosome pairing can be identified with a permutation function $\tau(i)$ of integers $i \in \{1,2,\ldots,N\}$, where N is the number of chromosomes in one cell and $\tau(i) = j$ if and only if chromosomes X_i and X_j form a pair. Obviously, $\tau(i) = j$, if and only if $\tau(j) = i$, and $\tau(i) \neq i$ for all i and j [5, 18]. In the pairing process, the whole chromosome set X can be represented by two subsets $\tilde{X}$ and $\bar{X}$, such that $\tilde{X} \cap \bar{X} = X$, and for $\forall i \in \tilde{X}$, there is $\tau(i) \in \bar{X}$. Let $P(X_i \cong X_{\tau(i)} \in \omega_k; f_i, f_{\tau(i)})$ be the posteriori probability that $X_i \cong X_{\tau(i)}$ given the features of X_i and $X_{\tau(i)}$, where $\cong$ is a binary relation such that $X_i \cong X_j$ if and only if two chromosomes X_i and X_j form a homolog pair and belong to the same class k. Then the problem of optimal pairing of chromosomes can be solved by maximizing the similarity measure function [5, 18]:

$$\tau_{\text{opt}} = \arg\max_{\tau} \prod_{i=1}^{N} P(X_i \cong X_{\tau(i)} \in \omega_k; f_i, f_{\tau(i)}) \tag{3}$$

over all pairing τ. Here, we assume that the random events $X_i \in \omega_k$ and $X_{\tau(i)} \in \omega_k$ are independent of each other for the given f_i and $f_{(i)}$; thus,

$$\begin{aligned} &P(X_i \cong X_{\tau(i)} \in \omega_k; f_i, f_{\tau(i)}) \\ &\quad = P(X_i \in \omega_k; f_i) P(X_{\tau(i)} \in \omega_k; f_{\tau(i)}). \end{aligned} \tag{4}$$

By taking logarithm of (3), we have equivalently,

$$\tau_{\text{opt}} = \arg\max_{\tau} \sum_{i=1}^{N} \log P(X_i \cong X_{\tau(i)} \in \omega_k; f_i, f_{\tau(i)}). \tag{5}$$

In order to solve the optimization problem of (5), we construct an undirected graph $G(X, E)$ with $|X| = N$ vertices and $|E| = M$ edges, where M is the number of chromosome pairs. Each vertex corresponds to a chromosome, and each edge to a pairing. The weight of such an edge is $\log P(X_i \cong X_{\tau(i)} \in \omega_k; f_i, f_{\tau(i)})$.

Given a candidate chromosome X_i, the likelihood that belongs to the class ω_k, due to its size, can be defined as follows [2, 4]:

$$P_{\text{size}}(X_i \in \omega_k; f_{\text{size},i}) = \frac{1}{\sqrt{2\pi}\sigma_k} \exp\left(-\frac{1}{2}\left(\frac{d_i - \mu_k}{\sigma_k}\right)^2\right) \tag{6}$$

where $d_i = \frac{|X_i|}{\sum_{i=1}^{N} |X_i|}$ is the normalized size of chromosome i, and μ_k and σ_k are the size mean and variance of class ω_k which can be calculated from the M-FISH image database.

Based on the characteristics of size and spectrum, the chromosome posteriori probability belonging to class k can be calculated based on the spectrum and size:

$$P(X_i \in \omega_k; f_{\text{size},i}, f_{\text{spectrum},i}) = P(X_i \in \omega_k; f_{\text{size},i}) \cdot P(X_i \in \omega_k; f_{\text{spectrum},i}). \quad (7)$$

$P(X_i \in \omega_k; f_{\text{spectrum},i})$ is the posteriori probability of the candidate chromosome belonging to class ω_k according to the average spectrum and can be calculated using Eq. (1).

4.2 Joint classification and pairing algorithm

Based on the previous analysis, the proposed classification-pairing algorithm by utilizing the spectral, texture and size characteristics of chromosome is summarized as follows:

1. Smooth each chromosome image channel by using a 5×5 Gaussian filter to remove the noise. Then create a binary mask of the DAPI channel by using Otsu's method [21], which is a nonparametric technique that provides a fast and simple way [22, 23] to select automatically a threshold value. This thresholding procedure is considered as the partitioning of the pixels of an image into two classes $H_0 = \{1, 2, \ldots, t\}$ (background) and $H_1 = \{t+1, t+2, \ldots, L\}$ (objects) where $L = 255$ is the total number of gray levels in the images.
2. Split the chromosomes into a series of homologous regions based on the multi-channel region fusion and classification algorithm proposed in Sect. 3, and assign a temporary class label to every homologous regions according to the maximum posteriori probability.
3. Combine the homologous regions to form several candidate chromosomes according to the spectral similarity and continuities. The candidate chromosomes' a posteriori probability belonging to class k can be calculated according to Eqs. (6) and (7).
4. For cells from male, the classification and pairing algorithm is used to choose the final segment results from potential chromosome sets. For cells from females, two dummy chromosomes are first constructed according to the a priori knowledge of relative size and spectrum of the X and Y chromosomes, and then the pairing algorithm is used to choose the final segmentation results from potential chromosomes set.
5. Final segmented chromosomes are the pairing result, corresponding to the minimal cost.

5 Experiment results

We have implemented the proposed joint chromosome classification and pairing algorithm based on the spectral, texture and geometric information and tested it on the ADIR M-FISH multi-spectral chromosome image database. The spatial resolution of the multi-spectral images is 517×645. Each pixel is represented as a six-element vector: five multi-spectral channels plus the grayscale DAPI channel. The database also includes a karyotyped "ground truth" image according to the ISCN (International System for Human Cytogenetic Nomenclature) for each M-FISH image, so the classification results can be easily evaluated with accuracy. In the experiments, we use the fuzzy c-means clustering method [24, 25] to acquire the homologous regions in the DAPI image (Haar wavelet is used to describe the local band patterns) and multi-spectral image. The number of clusters is 23 (for female) or 24 (for male). and we used the maximum flow method to solve the maximum-weight graph matching problem [26, 27]. Particularly, we used the function grMaxMatch in MATLAB Graph Theory Toolbox to get the optimal combination results.

The algorithm is tested on 183 images chosen from the ADIR M-FISH chromosome image database (for those abnormal cells with an odd number of chromosomes, a single chromosome is deleted by hand to make pairing work efficiently). Each image, together with classification results compared to the ground truth images, was analyzed and recorded. In all comparisons, we were somewhat lenient with both algorithms and accepted any segmentation that varied only slightly (segmentation error less than 1 %) from the ground truth image in the database. The results are presented in the following sections.

Figure 5 shows an example of image (A0502XY) segmented with the proposed method. Figure 5a shows the clustering map of the DAPI image, and Fig. 5b is the clustering map of the multi-spectral image. Compared with the hand-labeled karyogram result in Fig. 5d, the clusters (1), (2), (3) and (4) are well segmented properly. There were 200 clusters of touching and overlapping chromosomes in the testing experiment; 75 % of the clusters had two chromosomes, 20 % had three clusters, and 5 % had four or more chromosomes. The results of cluster separation by the proposed method are listed in Table 2. As seen from Table 2, 87 % of clusters are separated correctly. For clusters which contain more than two chromosomes, the proposed method shows better performance. The separation results obtained using different algorithms are shown in Table 3. Joint classification and pairing method correctly decomposed a much higher percentage of touches compared to the maximum likelihood (ML)-based joint segmentation-classification method [4] and geometric separation method [1]. If two chromosomes of the same class touch or overlap, there is no way to determine their boundary with multi-spectral information alone. Geometric information can be used to separate these chromosomes.

Table 2 Cluster separation results

Number of chromosomes in a cluster	Correct separation	Erroneous separation
2	93	7
3	85	15
≥4	83	17
Total	87	15

Table 3 Percentage of correct separation for various cluster types using different algorithms

	This paper's algorithm	ML method	Geometric separation
Touches	85	77	62
Overlaps	79	34	41

The segmentation accuracy of the ML method is inherently dependent on pixel classification accuracy. Pixel classification rates vary widely through the M-FISH image dataset [4]. All these factors influence segmentation performance by using the ML method. On the other hand, the geometric information is not so reliable in many cases to segment the touching and overlapping chromosomes, such as two chromosomes touching on their short side or long side and forming a long chromosome.

Figure 6 shows an example where two overlapping chromosomes have the same spectrum, just like cluster 1 in Fig. 5a. Spectral information alone cannot separate the two chromosomes, as there is textural difference between these two chromosomes. The texture clustering map can provide extra information to separate them.

Table 4 shows a comparison of several different classification algorithms. Schwartkopf et al. [5] proposed a joint segmentation and classification method employing pixel-by-pixel classification schemes; however, the classification will be dominated by noisy painting inhomogeneities. This is obvious in the misclassifications errors produced by the pixel-by-pixel algorithm. Choi et al. [28] and Wang et al. [10] also proposed the M-FISH chromosome classification and obtained good results on part of the ADIR M-FISH dataset. They also employed pixel-by-pixel classification schemes. Karvelis et al. [20] have given a multi-channel watershed-based method, where classification was based on the spectrum of all pixels belonging to a specific region. Region-based classification avoids these types of errors since pixels with similar spectral information contribute to the

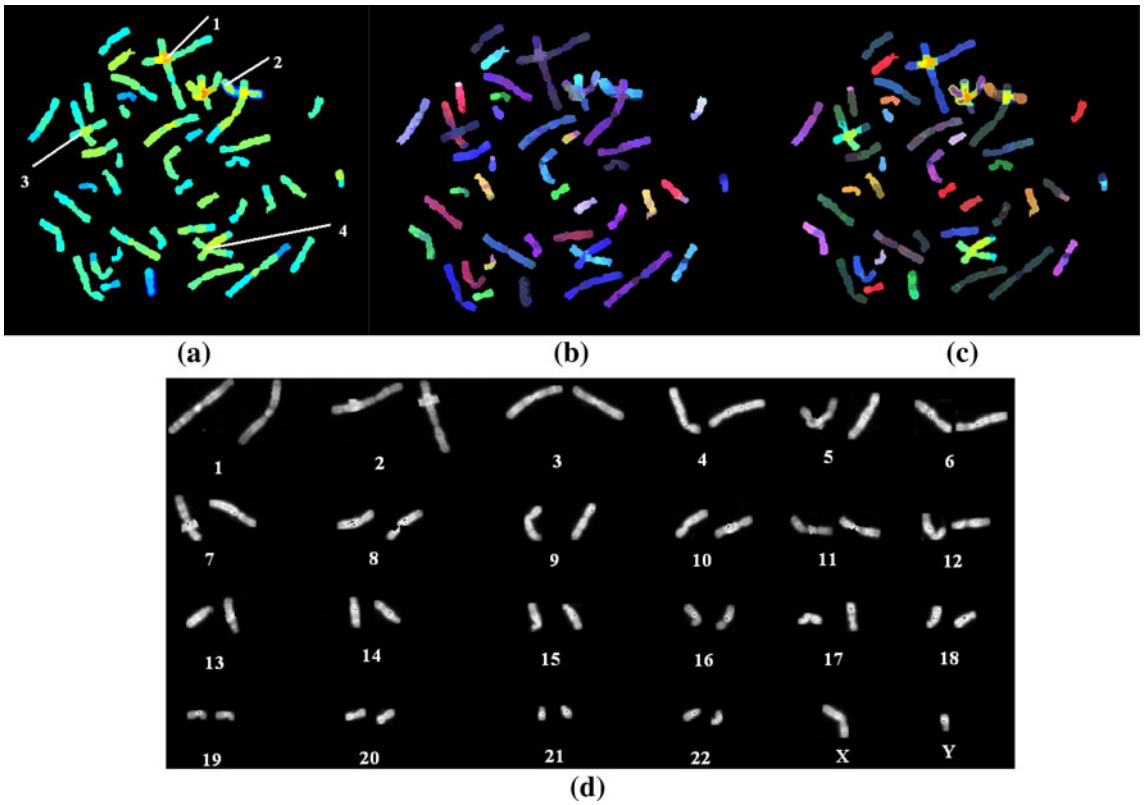

Fig. 5 Example of classification and pairing results with overlapping: **a** multi-spectral image of cluster, **b** multi-spectral clustering result, **c** texture clustering result, **d** classification and pairing result

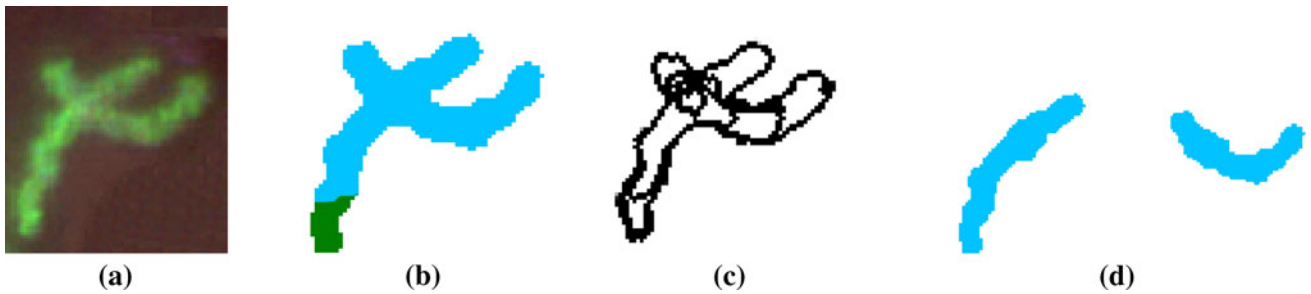

Fig. 6 Example of classification and pairing results: **a** the clustering map of DAPI image, **b** the clustering map of multi-spectral image, **c** fusion and classification result, **d** classification and pairing result

Table 4 Comparison of the proposed method and the other method that appeared in literature in terms of chromosome classification accuracy

Method	#Images	Average chromosome pixel classification (%)
Schwartkopf et al. [4]	183	68.0
Karvelis et al. [20]	183	82.5
Choi et al. [28]	10	97.1
Wang et al. [10]	5	87.5
This work	183	87.9

classification. Moreover, region-based classification provides better classification accuracy than the maximum a posteriori pixel-by-pixel classifier. But the main shortcoming of these methods is that they cannot effectively separate the touching/overlapping chromosomes with similar class label. The proposed algorithm utilizes the spectral and texture information, can separate the touching/overlapping chromosomes with similar class label, and it is also a region-based classification method. Although its average chromosome pixel classification accuracy is lower than the result in Choi et al. [28], the proposed algorithm is tested on 183 images while Choi et al. only tested their method on 10 images. For the results tested on 183 images, the proposed algorithm has the higher average chromosome pixel classification accuracy.

6 Conclusion

This paper presents a fully automated chromosome classification and pairing method for M-FISH images. The method utilizes a multi-channel region fusion and classification algorithm and a joint classification and pairing scheme. Initially, the chromosome image is decomposed into a set of homogeneous regions using the multi-channel region fusion and classification algorithm by utilizing the texture and spectrum information jointly. These regions of chromosomes are then classified using a region Bayes classifier. Then a graph-theoretical classification and pairing method is introduced to resolve any remaining ambiguity of the merging and classification process. To evaluate our method, we used a publicly available M-FISH database. The evaluation results demonstrated the superior performance of the proposed method to the previous methods in terms of classification and paring accuracy.

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