Proceedings of the 28th IEEE
EMBS Annual International Conference
New York City, USA, Aug 30-Sept 3, 2006

A Watershed Based Segmentation Method for Multispectral Chromosome Images Classification

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Abstract— **M-FISH (Multicolor Fluorescence In Situ Hybridization) is a recently developed cytogenetic technique for cancer diagnosis and research on genetic disorders which uses 5 fluors to label uniquely each chromosome and a fluorescent DNA stain. In this paper, an automated method for chromosome classification in M-FISH images is presented. The chromosome image is initially decomposed into a set of primitive homogeneous regions through the morphological watershed transform applied to the image intensity gradient magnitude. Each segmented area is then classified using a Bayes classifier. We have evaluated our methodology on a commercial available M-FISH database. The classifier was trained and tested on non-overlapping chromosome images and an overall accuracy of 89% is achieved. By introducing feature averaging on watershed basins, the proposed technique achieves substantially better results than previous methods at a lower computational cost.**

I. Introduction

CYTOGENETICS is a high complexity area of the clinical diagnostic laboratory. Cytogenetic technologists study the hereditary material at the cellular level by examining the structure and behavior of chromosomes. Chromosomes are the condensed form of the genetic material and their images taken during cell division are useful for diagnosing genetic disorders and for studying cancer [1].

Normal cells contain 46 chromosomes which consist of 22 pairs of similar, homologous chromosomes and two sex-determinative chromosomes (XY: male and XX: female). The procedure of assigning every chromosome to each class is called Karyotyping [1]. However, many images often have to be inspected and since visual inspection is time consuming and expensive, many attempts have been made to automate chromosome image analysis.

In an attempt to ease the process of imaging the chromosomes a newly developed cytogenetic technique was proposed [2]. In this technique all chromosomes are labeled with 5 fluors and a fluorescent DNA stain called DAPI (4',6-Diamidino-2-phenylindole). DAPI attaches to DNA and thus labels all chromosomes. The other five fluors attach to specific sequences of DNA in a way that each class of chromosome absorbs a unique combination of fluors. So at least $N = 5$ fluors are needed for combinatorial labeling to uniquely identify all 24 chromosomes. Using this combinatorial labeling, it is possible to determine the most likely chromosomal origin at every point in the image.

M-FISH images are captured with a fluorescent microscope with multiple optical filters. Each of the fluors is visible in one of the spectral channels in a way that an M-FISH image consists of six images and each image is the response of the chromosome to the particular fluor (Fig. 1).

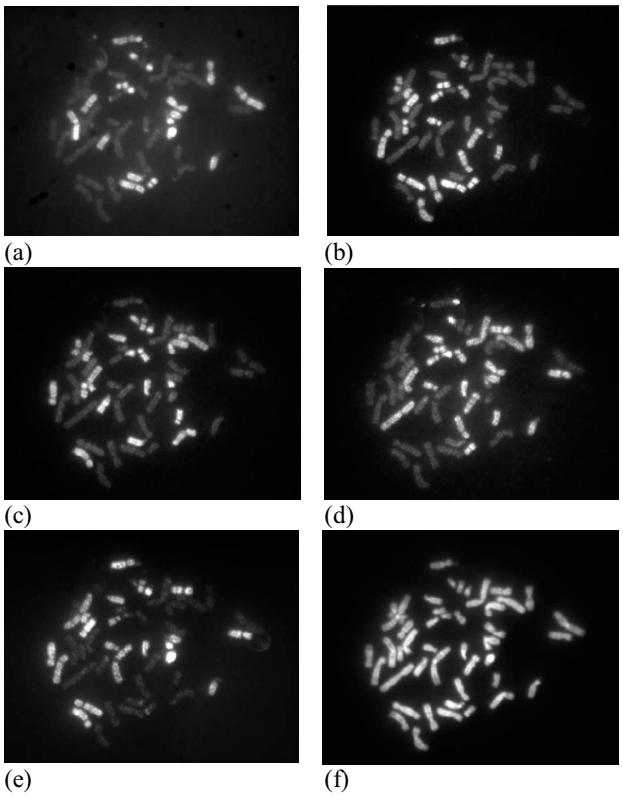

Fig. 1. Six channel M-FISH image data: (a) Aqua fluor, (b) Far red fluor, (c) Green fluor, (d) Red fluor, (e) Gold fluor, and (f) DNA DAPI stain.

Semiautomated analysis of M-FISH images was first introduced in the mid 90's [3]. The DAPI channel was used to create a binary mask. Then for each pixel of the mask a threshold was applied in order to detect the presence or absence of a fluor to that pixel. Each pixel class was

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determined by comparing the response of the combined fluors to that of a labelling table.

The procedure was automated [4, 5] by modelling the task as a 5 feature 24 class pattern recognition problem. An image tessellation algorithm was first introduced and a recursive region merging algorithm was applied based on the average colour vectors of each area. The classification problem has been also modelled as a 6 feature 25 class problem adding the background as an extra class [6, 7].

Recently, a method for joint segmentation and classification of chromosome M-FISH images was presented [8]. They introduced a probabilistic model of M-FISH chromosomes which allows for simultaneous segmentation and classification. The additional information provided by multiple spectra in chromosome images made it feasible to distinguish chromosomes that overlap and touch within clusters [9].

Although the above methods have achieved satisfactory results improvements can be made. Most of the methods use pixel-by-pixel classification which can produce noisy results [4]. Indeed, for this type of classification, performances can vary significantly through the M-FISH image dataset. Accuracies above 90% have been reported in some images [6-8] but the average classification accuracy for the whole set was only 68% with standard deviation 17.5% [9].

In this paper a method for the classification of multispectral chromosome images is presented. The method uses the watershed transform to produce an image tessellation of the homogenous regions of the image. These areas are then classified using a Bayes classifier. The proposed method is innovative since it makes use of the characteristics of each watershed area in order to classify a pixel. This reduces the computation time significantly. Furthermore the proposed method reported marginally better results than a pixel-by-pixel classification method.

II. Materials And Methods

Our proposed method consists of a number of steps as it is shown in Fig. 2. The first step is the computation of the gradient magnitude of the grayscale DAPI channel. The watershed transform is applied in the next step and a large number of primitive homogeneous regions (oversegmentation) is produced. A binary mask of the DAPI channel is computed in order to further reduce unwanted areas. Finally, for each area a 5 feature vector is computed, each feature representing the average intensity value of each channel.

A. *Image Segmentation*

The goal of this stage is to create a mask of pixels to be classified. As mentioned before, the DAPI stain labels all the chromosomes and thus this image can be used for the segmentation. First the image gradient magnitude $G^I = \|\nabla I\|$ of the initial image I is computed.

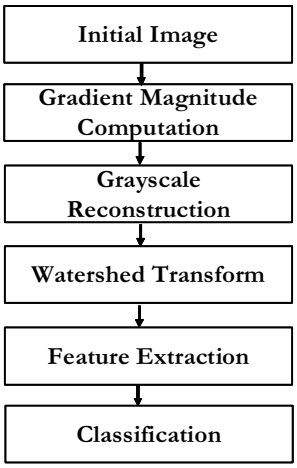


Fig. 2. The proposed classification method.

Due to the high sensitivity of the watershed algorithm (WT) to the gradient image intensity variations, the WT produces image partitions containing a large number of regions.

A very efficient method to reduce the number of minima in a grayscale image is grayscale reconstruction [10]. Let I and J be two grayscale images taking their values from the discrete set $\{0,1,\ldots,L-1\}$ where L is the number of intensity levels, such that $J \le I$ (i.e., for each pixel $p \in I$, $J(p) \le I(p)$). The grayscale reconstruction $\rho_I(J)$ of (mask) I from (marker) J is:

$$\forall p \in I,\ \rho_I(J)(p) = \max\left\{k \in [0, N-1] \middle| p \in \rho_{T_k(I)}(T_k(J))\right\}, \quad (1)$$

where

$$T_k(I) = \{p \in I \,|\, I(p) \ge k\}. \quad (2)$$

In order to reduce the number of minima of the gradient magnitude we apply the greyscale transform as follows:

$$R_h(G) = \left[\rho_{[G]_c}([G]_c - h)\right]_c, \quad (3)$$

where G is the gradient magnitude image, $R_h(G)$ the grayscale reconstructed gradient magnitude, $[\cdot]_c$ denotes the complement operator and $h \in \Re$.

The computation of the watershed transform (WT) [11] is the next step of our method. The watershed transform is a popular segmentation method originated in the field of mathematical morphology. The image is considered as a topographical relief, where the height of each point is related to its grey level. Imaginary rain falls on the terrain. The watersheds are the lines separating the catchment basins.

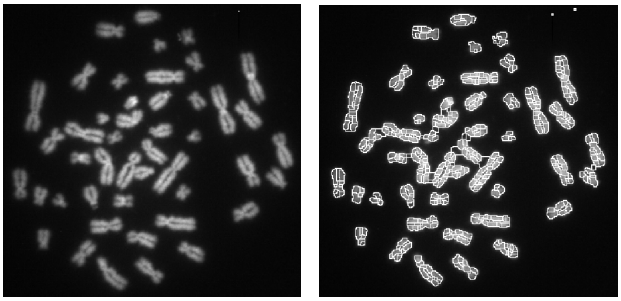
The output of the WT is a tessellation T_K of the image into its different catchment basins, each one characterized by a unique label:

$$T_K = \{T_1, T_2, \ldots, T_K\}, \quad (4)$$

where K is the number of regions. Each region

$T_i, i \in \{1,2,\ldots,K\}$ represents a regional minimum[1] with its associated catchment basin.

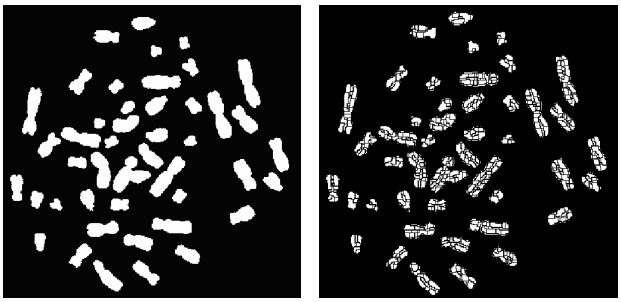
Only the pixels belonging to the watershed transform are assigned a special label to distinguish them from the catchment basins. The application of the WT to the grayscale reconstructed gradient magnitude image is illustrated in Fig. 3.



(a) (b)

Fig. 3. Watershed segmentation applied to the grayscale reconstructed gradient image: (a) Initial image, (b) The image after watershed segmentation.

In order to further reduce unwanted minima outside the region of chromosomes the initial image is converted to binary using a well known automated threshold selection process [12] (Otsu's method). In this way a binary mask is created and is superimposed on the tessellation so that minima outside the area of chromosomes are eliminated. Elimination of unwanted minima is shown in Fig. 4.



(a) (b)

Fig. 4. (a) Binary image, (b) The image after the binary mask superimposed on watershed segmentation.

B. *Feature Extraction and Classification*

The aim of this stage is to classify each segmented area produced by the watershed transform. For each segmented area produced by the watershed transform a five feature vector $x \in \Re^5$ is created. Each feature represents the average intensity value for each of the 5 channels.

[1] A regional minimum M of a grayscale image I at intensity level h is a connected set of pixels with intensity h, such that it is impossible to reach a pixel of intensity h' without having to pass from a pixel of intensity h'', where $h' < h < h''$.

For the classification step we have chosen to implement a Bayes classifier in order to compare the proposed methodology to other pixel by pixel classification methods [6-8] which use the same classifier. Our objective is to classify the 46 human chromosomes which consist of 22 pairs of similar chromosomes and 2 sex determinative chromosomes. So the number of classes is 24 ($C = 24$).

Let $x \in \Re^5$ denote the feature vector of each segmented area, and $P(c_i)$, $i = 1,2,\ldots,C$ the *a priori* probability that a feature belongs to class c_i. Let $p(x \mid c_i)$ denote the class conditional probability distribution function. It represents the probability distribution function, for a feature vector x given that x belongs to class c_i. Let also $P(c_i \mid x)$ denote the *posterior* probability which represents the probability that the feature vector belongs to class c_i given the feature vector x. Then using Bayes theorem:

$$P(c_i \mid x) = \frac{p(x \mid c_i)p(c_i)}{\sum_{i=1}^{24} p(x \mid c_i)p(c_i)}. \quad (5)$$

We have used the Gaussian probability density function in order to model the distribution:

$$p(x \mid c_i) = \frac{1}{(2\pi)^{d/2}\left|\Sigma_i\right|^{1/2}} \exp\left(-\frac{1}{2}(x-\mu_i)^t \Sigma_i^{-1}(x-\mu_i)\right), \quad (6)$$

where x is the d feature vector ($d = 5$), μ_i is the mean vector of the class c_i, Σ_i is the $d \times d$ covariance matrix of the class c_i, $\left|\Sigma_i\right|$ and Σ_i^{-1} are determinant and inverse, respectively. Also $(x-\mu_i)^t$ denotes the transpose of $(x-\mu_i)$.

To classify any given test sample described by the feature vector x, we calculate $P(c_i \mid x)$ for each class c_i. The class, to which the sample belongs, is given by the Bayes Decision Rule:

$$Decide\ c_i\ if\ P(c_i \mid x) > P(c_j \mid x),\ \forall j \neq i. \quad (7)$$

The prior class probabilities $p(c_i)$ are computed from the training set as follows:

$$p(c_i) = \frac{\#of\ pixels\ belonging\ to\ class\ i}{total\ \#\ of\ pixels}. \quad (8)$$

Using the above equation, large chromosomes have higher prior probability than smaller ones.

III. DATASET & RESULTS

We have evaluated our methodology using the ADIR [13] commercial database which contains M-FISH images. For each set of M-FISH images the database contains also a labeled class-map image in which each pixel is labeled according to the class to which it actually belongs. This image is used to determine the accuracy of the classification techniques. Seventeen images were randomly chosen, from

which two were used for training and the remaining for testing.

The performance was measured by means of accuracy:

$$Acc = \frac{\#of\ correctly\ classified\ pixels}{total\ \#of\ pixels}. \quad (9)$$

Finally, receiver operating characteristics (ROC) analysis was performed for the classifier with the best performance, following the *class reference* formulation [14]. In order to compute the multi-class AUC_{total} the following formula was used :

$$AUC_{total} = \sum_{i=1}^{C} AUC(c_i) \cdot p(c_i), \quad (10)$$

where $AUC(c_i)$ is the area under the class reference ROC curve for class c_i, C is the number of classes ($C = 24$) and $p(c_i)$ is the ratio of the test pixels belonging to class c_i of each image, to the total number of test pixels.

The classification results of our methodology are shown in Table I. The results using pixel-by-pixel classification are given also in Table I.

TABLE I
COMPARISON OF THE PERFORMANCE OF THE TWO METHODS

	Pixel–by-Pixel Classification		Watershed Area Classification	
#Image	***Acc* (%)**	AUC_{total}	***Acc* (%)**	AUC_{total}
1	93.84	0.990	96.11	0.996
2	86.40	0.987	97.11	0.995
3	82.20	0.978	95.81	0.994
4	72.70	0.933	85.30	0.953
5	80.60	0.965	93.78	0.989
6	74.50	0.952	91.38	0.990
7	61.50	0.894	72.50	0.931
8	66.10	0.916	74.30	0.953
9	82.00	0.964	91.44	0.987
10	83.30	0.969	93.77	0.995
11	77.80	0.956	91.57	0.985
12	64.10	0.923	82.00	0.965
13	65.50	0.938	86.90	0.960
14	85.30	0.985	96.21	0.996
15	82.30	0.965	94.75	0.991
Overall	**77.21 ± 9.5**	**0.95 ± 0.03**	**89.53 ± 7.9**	**0.98 ± 0.02**

IV. DISCUSSION

An automated method for the classification of multispectral chromosome images based on the watershed transform has been presented. Initially, the chromosome image is decomposed into a set of primitive homogeneous regions. Each segmented region is then classified using a Bayes classifier. Our methodology has been evaluated using the commercially available M-FISH database and an overall accuracy of 89% was reported.

Our approach performs better in terms of accuracy, when compared with a pixel-by-pixel classification method (Table I). In addition, the proposed approach reduces the computation time since only watershed regions have to be classified and not individual pixels. More specifically, an average number of 500 ± 140 watershed regions have to be classified whereas in pixel-by-pixel classification, this number reaches up to 11469 ± 1977 pixels. Furthermore, pixel-by-pixel classification can produce noisy results, since some isolated pixels and small segments are misclassified. Our method deals with the above aspect effectively using region classification. Indeed, for pixels around the perimeter of the chromosome, classification errors were common [6-8]. Our approach can handle it, since these pixels belong to larger areas, which are classified correctly.

Future work should focus on the evaluation of our method in a larger set of images. Also, a more efficient technique to address overlapping chromosomes is under consideration.

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