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Automated Identification of Polytene Chromosomes Banding Pattern using Image Processing Techniques

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Abstract

An automated method has been developed to identify the dark bands and light-inter bands in the Polytene chromosomes microscopic images using the digital image processing algorithms. Polytene chromosomes are specific interphase large chromosomes which have alternation of dark bands and lighter inter bands morphology. Analysis of these banding patterns is very important in many structural and functional biological researches such as chromosomes mapping, identify small chromosome mutations, identify taxonomic species, and classify the sibling species.

The algorithm of banding pattern identification and analysis follows a step-by-step strategy and the overall system is modularized into three stages: preprocessing, segmentation and feature extraction. The preprocessing stage contains the techniques to prepare the image for segmentation; the techniques deal directly with the raw, possibly noisy, pixel values with de-noising and contrast adjustment. The segmentation stage finds and establishes outlines of specific dark/lighter-inter bands using rolling ball algorithm and automatic thresholding techniques. The feature extraction stage obtains semantics of the banding pattern and useful quantitative parameters from the segmented bands. In this work, some samples of straightened Polytene chromosome microscopic images were analyzed with satisfactory results.

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Keywords: Polytene Chromosome, Banding Pattern, Rolling Ball

Introduction

Biological research immensely depends upon the correct identification of organisms. In addition, to develop essential tools for systematic analysis is highly useful in biological studies of diverse nature. This is very much true in the case of Polytene chromosome analysis because Polytene chromosomes are central objects for the analysis of many features of chromosome organization and the genome. Polytene chromosomes consist of thousands of DNA strands and are seen to have distinct dark and lighter banding patterns [1]. Polytene chromosome banding pattern analysis is a standard procedure to classify the species and other biological research purposes. Especially, Polytene chromosome banding pattern is widely used to identify sibling species in many species complexes [2]. Various features of the banding patterns of Polytene chromosomes were proposed and used for classification by the researchers manually [1,3]. Efficient automated object and pattern identification algorithms and software tools are becoming increasingly important in recent years as the difficulties in the manual methods. However, very limited studies have been published in the literature such as automate the classification of human chromosome [4], straighten human chromosome [5,6], and centromere and length detection in straightened human chromosome [7], as the quality of the microscopic images and the chromosome preparations are still in the developing stage.

An automated Polytene chromosome banding pattern identification system has been proposed here using microscopic image analysis techniques. To our knowledge, this is the first attempt to detect the bands and measure the bands in a Polytene chromosome automatically. To achieve this, image processing algorithms were placed at three levels. At the lowest level, methods deal with preparing the image for segmentation. In the middle level, algorithms are utilized low-level results for further means with background removing and bands segmentation. At the highest level, techniques attempt to extract the pattern and properties of bands.

Methodology

Data: A number of Polytene chromosome samples from various species prepared under the same conditions and straightened microscopic images is typically assumed to be in the white background an accurate representation of the chromosome [5].

Dark-Band Identification

The fully automated dark band identification consists of the following steps:
STEP 1 (Grayscale): The microscopic straighten colour Polytene chromosome images have been converted into grayscale images initially using Z=0.299 * R + 0.587 * G + 0.114 * B as acquired colour images are not suitable for image processing steps of band identification.
STEP 2 (De-noising and Smoothing): A Gaussian smoothing 2-D convolution operator algorithm [8] is applied on grey scale image to reduce image noise, smooth the image and preserve edges which is suitable for applying the rolling ball algorithm later. The degree of smoothing is determined by the standard deviation of the Gaussian, which was obtained using the training of some image samples in the identification.
STEP 3 (Complement): Smoothed image (I) has been transferred to complement of the image J (=255-I) as for finding the dark bands in the white background.
STEP 4 (Contrast Adjustment): The negative image from the previous step underwent a contrast adjustment procedure to boost the separation between the darkest and brightest areas of the image. The contrast adjustment intensity values were automatically defined from the histogram of the image.
STEP 5 (Background Removal): The background intensity level of images, on which the dark bands appear, is not uniform over an image and varies between images. Thus we must remove the background from the image before taking the thresholding for segmentation. Rolling ball algorithm [9] used here to find the image's smooth continuous background. Consider the 2D grayscale image from the previous step has a third (height) dimension defined by the intensity value at every point in the image. The radius of the "ball" was determined by experimenting with the sample images. The ball is moved along each scan line of the image and background is determined during the process. The identified background was subtracted from the image.
STEP 6 (Threshold): An automatic threshold is found for the filtered image using Otsu's method [10]. This threshold is applied to the image in order to separate the dark bands from the background.
STEP 7 (Morphological Operations): In this stage the binary morphological operations 'Dilation' and 'Erosion' are applied in the order to the segmented binary image to remove the small blobs in the background and to connect the breakdown band components together.
STEP 8 (Complement): The segmented bands have been reversed to appear the dark bands in the white background.

Lighter-Inter Band Identification

The fully automated lighter-inter band identification consists of the following step:
STEP 9: Combining the image from the STEP 8 of dark band identification and the original grey scale image, we first segmented the rest of the areas of the original image by taking out the identified dark band pixels. Each horizontal line the border pixels were removed comparing with the number of pixels in the average width of the chromosome.

Combining Dark and Lighter-Inter Bands

Identified dark and light-inter bands were combined together for analyzing the band pattern and feature extraction. Each band is labeled according to the determined components. Band pattern is defined for the particular chromosome and quantitative values (area, height, and width) were measured for each band.

Results and Discussion

Dark Band Identification

The Figure 1 illustrates the dark band identification for a chromosome image described in the methodology section. Dark bands are extracted from the original image clearly and they are ready for the feature extraction step.

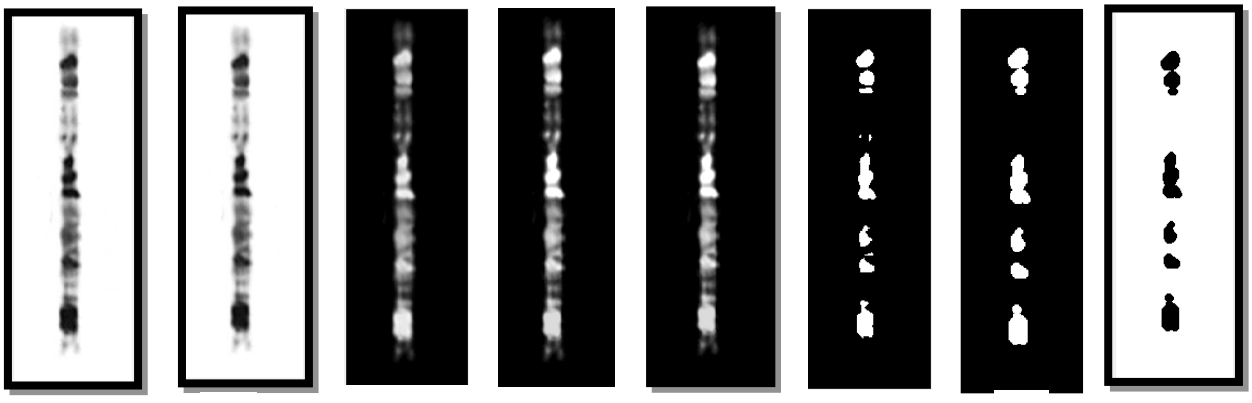


Figure 1. Dark band identification (a) Original gray scale Polytene chromosome (Result of STEP 1) (b) Result of STEP 2 (c) Result of STEP 3 (d) Result of STEP 4 (e) Result of STEP 5 (f) Result of STEP 6 (g) Result of STEP 7 (h) Result of STEP 8

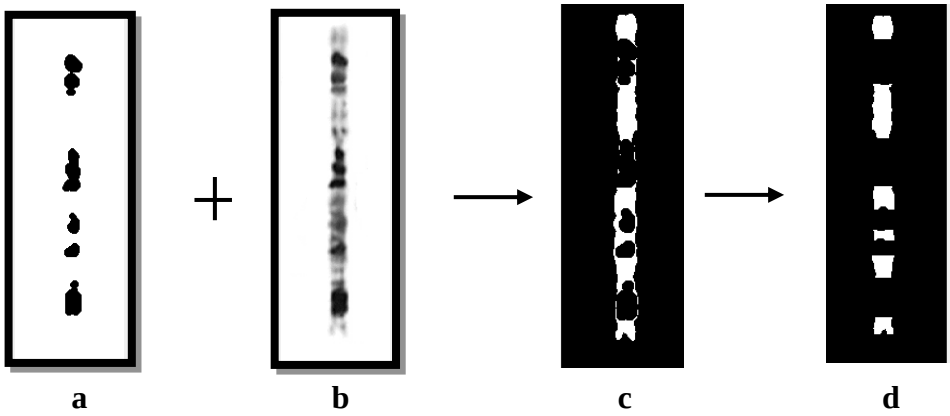


Figure 2. Lighter-band identification (a) Dark bands from the STEP 8 (b) Grey scale image from the STEP 1 (c) Segmented the rest of the areas from the dark bands from the STEP 9 (d) Final lighter-inter bands

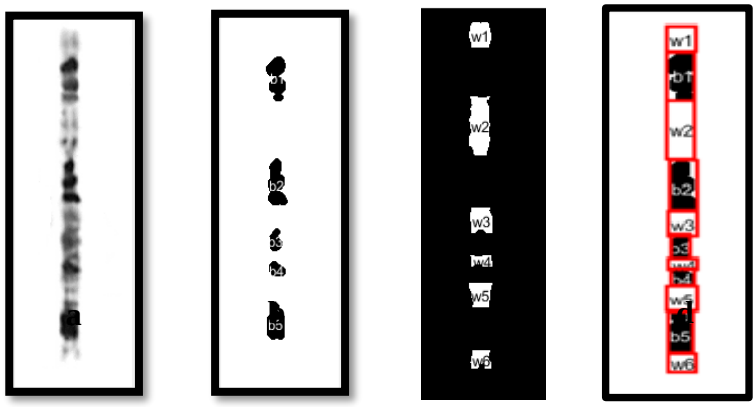
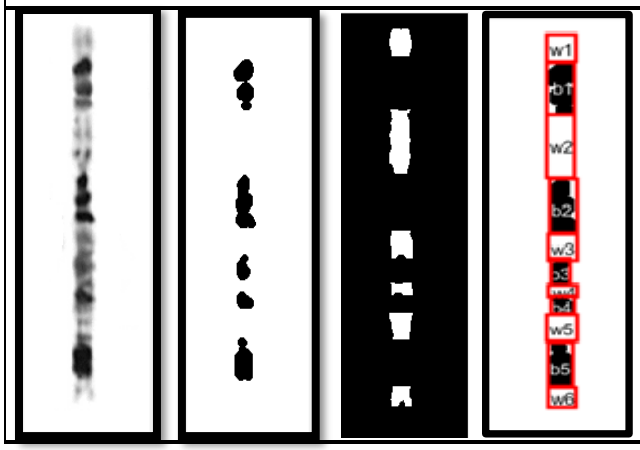
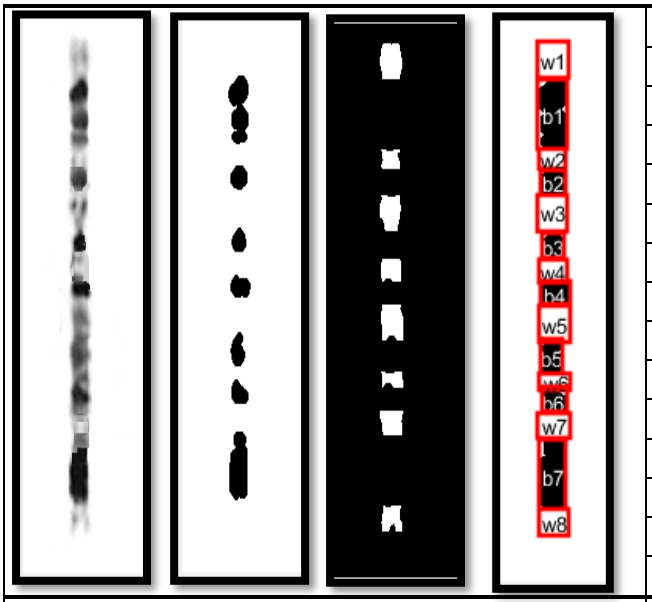


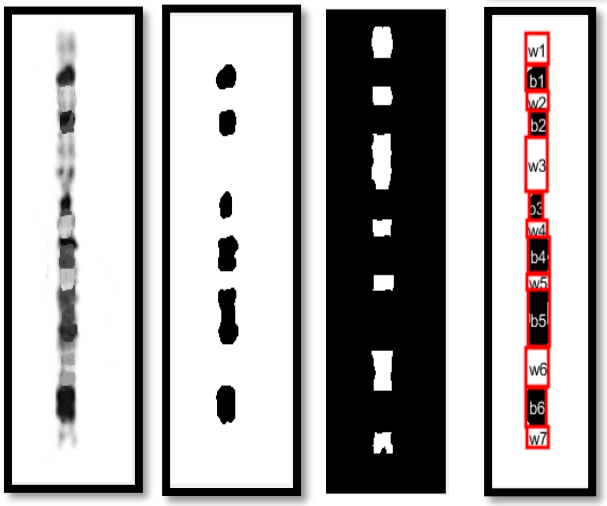
Figure 3. Combining ad labeling dark bands and lighter-inter bands (a) Grey image from the STEP 1 (b) Dark bands from the STEP 8 (c) Lighter-inter bands from the STEP 9. (d) Combined dark and lighter-inter bands.

Table 1. Banding patterns of three different chromosomes and the properties (Area, Height and Width in number of Pixels) of each dark band (b) and lighter-inter band (w).

Chromosomes	Bands	Area	Height	Width
	w1	390	22	20
	b1	521	41	19
	w2	821	51	19
	b2	560	35	17
	w3	360	21	20
	b3	197	21	19
	w4	165	10	21
	b4	172	14	13
	w5	392	22	20
	b5	466	43	16
	w6	247	16	19



w1	346	22	19
b1	575	42	19
w2	180	12	17
b2	178	15	16
w3	390	22	20
b3	158	15	14
w4	235	14	18
b4	206	14	19
w5	392	22	20
b5	191	21	14
w6	157	10	20
b6	206	15	16
w7	281	15	21
b7	606	42	17
w8	242	16	19



w1	390	22	20
b1	265	18	19
w2	226	13	19
b2	262	19	16
w3	640	39	19
b3	190	20	12
w4	203	12	18
b4	427	26	19
w5	195	11	20
b5	609	41	19
w6	470	28	20
b6	402	28	18
w7	231	15	19

Light Inter Band Identification

The Figure 2 illustrates the lighter inter band identification from the STEP 8 of the dark band identification algorithm as described in the methodology section and the original greyscale image. Lighter inter bands are segmented and they are ready for the feature extraction step.

Semantics of Dark and Light-Inter Bands

Combined dark bands and lighter-inter bands are labeled and identified the banding pattern of the chromosome, which is demonstrated in Figure 3. The complete process of three different species Polytene chromosomes and their banding pattern and their properties are shown in Table 1. The number of dark bands and lighter-inter bands are different in each chromosome and the size of the bands also vary for each band and chromosomes. The banding pattern and the properties are strongly related to their morphological and physiological similarities and differences.

Automatic segmentation of dark and light-inter bands of Polytene chromosome is described in this work and the results show very promising. In our future work, we have planned to straighten the curved Polytene chromosomes and taking more samples to be analyzed to classify the species.

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