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Human chromosome classification based on local band descriptors

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Abstract: In this paper several new techniques for automated chromosome analysis are described: one for piecewise-linear chromosome stretching and projection, two for accurately localizing the centromere and one for two-dimensional local band pattern description. A classification procedure is described that is based upon local band descriptors. Classification results obtained with this method are compared with results obtained with the global band description method (WDD functions). Data sets from two different laboratories are used to investigate the influence of the preparation. Results show the suitability of the local description method in its ability to visualize the image processing technique at the level of the chromosome image.

Key words: Chromosome classification, band descriptors, chromosome stretching, centromere finding, comparative study.

1. Introduction

Automated human chromosome classification has a 20 year history. As early as the mid-sixties, automated analysis was initiated at several laboratories (Rutovitz (1968), Neurath et al. (1965), Ledley et al. (1965)). The early optimism, of automating a task, which seemed trivial as a trained observer can perform it in 30 seconds, was not followed by decisive results. Many other research groups joined the field but progress beyond the point where 70–80% good classifications were scored was cumbersome. Partial explanation of the underestimated complexity of the problem is to be found in the fact that chromosomes are not as 'stable' objects as was first

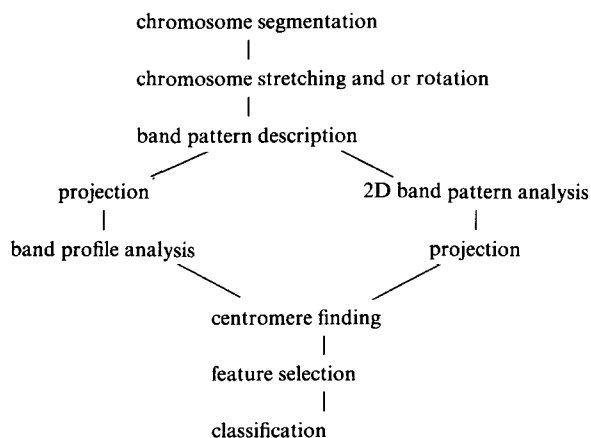
assumed. Lundsteen et al. (1980), report that using band staining techniques, not more than 58% of all bands supposedly present are found in reality. In addition, the band pattern (when completely present) shows a great variation in contraction from chromosome to chromosome and within and between the two legs. This makes normalization difficult. The variability within one chromosome type is illustrated in Figure 1, showing chromosome 2 originating from different metaphases. Apart from difficulties associated with staining techniques, complications arise from chromosomes having a random orientation and being possibly bent, overlapping and touching each other. Finally, from a classification perspective the number of classes is



Figure 1. Chromosome number 2 from various metaphases.

high, certainly when taking the variability of the appearance of the individual class members into account, Smeulders (1978). So the problem could only be solved when extensive, well-documented databases and robust features became available, Lundsteen (1980), van der Ploeg (1974).

The analysis of single chromosome images includes the following steps:



These topics except for the chromosome segmentation are covered in the next sections, although not in the indicated order. Special attention is given to the band pattern description which involves either projection of the chromosome image into a band

profile and subsequently the analysis (global band description), or first the analysis of the bands in the 2D chromosome image prior to the projection (local band description). The chromosome segmentation comprising the metaphase-finding step and the actual determining of the chromosome region(s) falls outside the scope of this paper.

2. Existing methods

2.1. Projection

The bands are, in principle, perpendicularly to the long axis of the chromosome, resulting in a sequence of bands along this axis. In analysis, the two dimensional image of the bands may therefore be reduced to a one dimensional profile. The projection may be computed at different stages in the chromosome analysis. Several factors influence the accuracy of projecting the two dimensional image onto a one dimensional axis. First, unequal stretching of corresponding places on the chromatids smears out the projection of the bands on the axis, see Figure 2. Secondly, in practice, the bands of course deviate from the ideal rectangular shape and may have protrusions along the chromosome border. Projecting these irregularly shaped bands

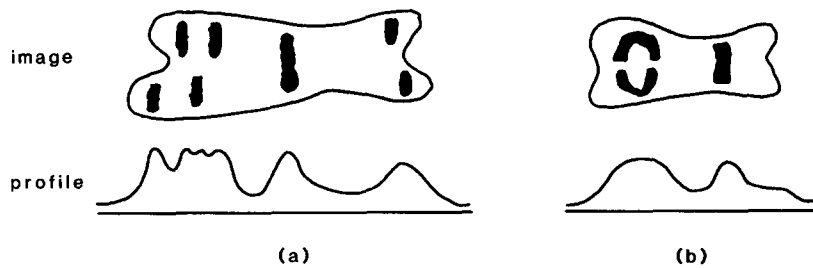


Figure 2. Potential problems occurring when projecting: (a) unequal stretching of the chromatids leads to smearing of bands, (b) protrusions may result in artificial fusion of bands.

without precaution causes undesirable results. Thirdly chromosomes have a random orientation with respect to the scanning grid. Due to the non-isotropic property of the sampling grid the pixels of the projection are obtained by sampling or interpolation of the scan grid pixels. The resulting requantization introduces additional quantization errors depending on the orientation of the object. Groen et al. (1976), showed that the requantization error may be reduced to an acceptable value ($< 2\%$) if the scanning grid is sufficiently fine or if an appropriate interpolation technique is used. Fourthly, apart from its orientation a chromosome may be bent. This problem is solved by letting the axis of projection follow the bending, introducing requantization errors of course, Groen et al. (1979), Selles et al. (1976), Merritt (1983).

2.2. Centromere finding

The determination of the centromere position is an important step in processing chromosome images. In addition to being a guide in establishing the proper orientation up or down, the centromere position is an important feature in chromosome classification. Several methods have been introduced. Gallus et al. (1970), detects the centromere position by locating the maximum concavity in the chromosome contour, Piper (1981), by laying a convex hull around the object image and Ueberreiter (1982), using a topological method.

2.3. Band pattern descriptions

Descriptors of band patterns can be divided into two categories: the global descriptors and the local ones. Global descriptors result from an overall

analysis, not localizing bands as such, Casperson et al. (1970), Piper et al. (1980), Oosterlinck (1977). In the local band descriptors bands are segmented first and thereafter features are measured, Granlund (1973), (1976), Vanderheydt et al. (1980), Lundsteen et al. (1981).

Local analysis offers the advantage that at a certain stage the success of the operation may be evaluated, and the analysis of the bands may be named individually. Thus, band description may be expressed in a format comparable to the one used by a cytogeneticist. Since local band description methods require the detection of bands in the 2D-chromosome image, in this way projection errors can be avoided. In local analysis the number of (relevant) features varies from class to class. This property facilitates an easy adaptation of the set of features to identify a specific chromosome class but, at the same time imposes a relatively complex classification scheme.

With global descriptors the number of features is fixed a priori. Global descriptors are usually less ad hoc and more mathematically based. The global description with its formal structure has fewer degrees of freedom and is thus more rigid. Therefore, global descriptors lead to a relatively simple classification scheme.

2.4. Classification results

In chromosome classification a superior result is claimed by Granum et al. (1981), who used a Haar-like set of functions, called WDD functions (Weighted Density Distribution) shown in Figure 3. The chromosome profile is correlated with these functions to produce the global features. Some of these functions are specifically tailored to the cen-

tromere position. The results obtained with this global method was used as a reference for the local method to be described here.

3. Proposed techniques

3.1. Projection and stretching method

When a chromosome is not straight it is usually 'cracked' rather than bent. Therefore, the employment of a piecewise-linear (PWL) approximation is preferred over the use of the existing polynomial approximation techniques.

First, the rough orientation of the principal axis of the chromosome is calculated from the second order moments of the chromosome grey-values. Thereafter, points on the middle axis of the chromosome are found by computing the middle of the chromosome in columns perpendicular to this principal axis (Figure 4a). As the columns are perpendicular to the axis and not to the chromosome, artifacts may occur at the tips. Therefore, the tips are temporarily disregarded in the PWL approximation.

To compute the PWL approximation to the middle axis the following procedure is followed. Starting with the line-segment between the endpoints of

the middle axis, the point on the middle axis maximally distant from this line-segment is calculated. When this distance is larger than a certain threshold the line-segment is split into two line-segments with the breakpoint at the calculated point. The procedure is repeated until all distances are smaller than the threshold. (See Figure 4b.) The chromosome is now stretched by requantization of the object along lines perpendicular to the PWL axis. Due to the sudden change in direction of the PWL-axis at a breakpoint, ambiguity in requantization may arise around breakpoints of the axis. In the neighbourhood of a breakpoint, points within the chromosome area may lie on two requantization lines. To avoid this ambiguity, the change in direction in a breakpoint is done in some n steps. This results in requantization lines around a breakpoint which are not perpendicular to the PWL approximation. This procedure is illustrated in Figure 4c. The value of n is chosen in such a way that the intersection of successive requantization lines lies outside the chromosome boundary. In the appendix it is proven that the minimal distance of the intersection of two requantization lines to the PWL axis is in approximation:

$$h \cos^2(\varphi/2)/\sin(\varphi/n) > w/2$$

where h is the grid constant and φ is the angle be-

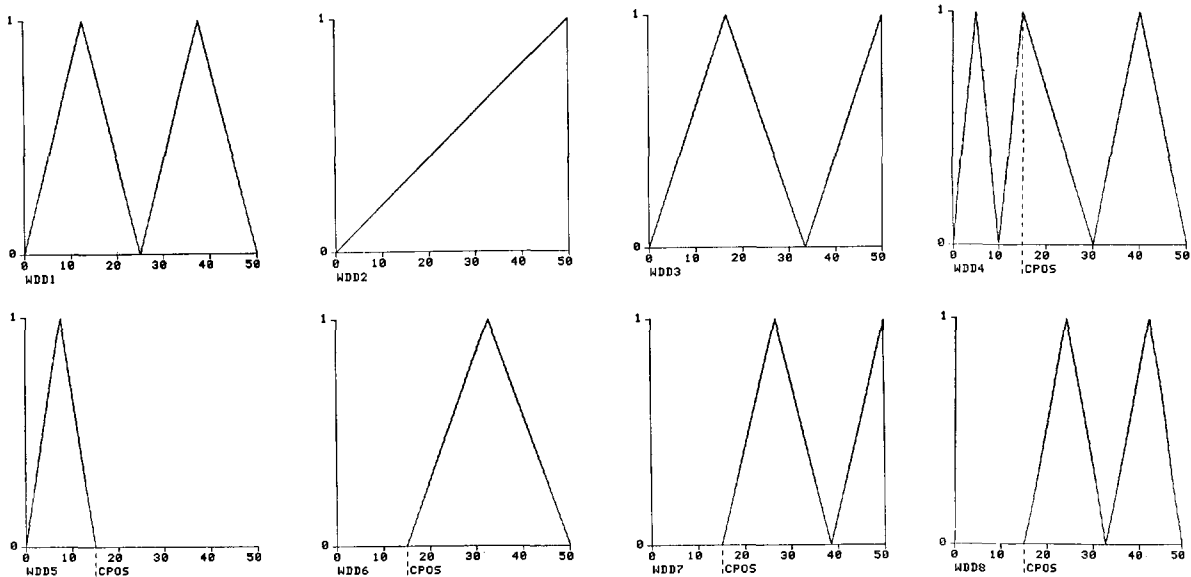


Figure 3. WDD function definitions.

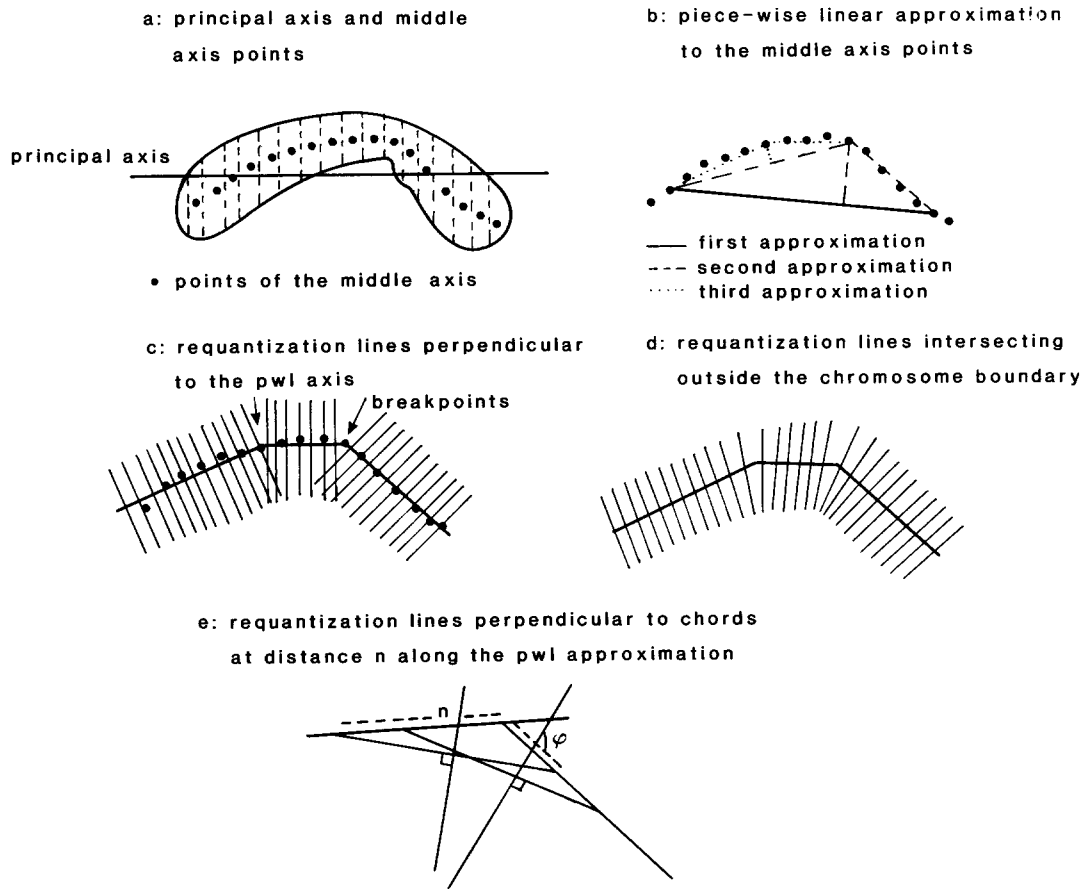


Figure 4. Chromosome stretching and rotation. (a) The middle of the chromosome is computed in columns perpendicular to the principal axis. (b) A piecewise-linear approximation is fitted to these middle axis points. (c) Requantization lines perpendicular to this PWL approximation would cross inside the chromosome region. (d) To prevent this, adjusted requantization lines are taken.

tween two successive parts of the PWL approximation. (See Figure 5.) This distance must be larger than $w/2$, where w is the chromosome width. Thus

$$n > \varphi / \sin^{-1}(2h \cos^2(\varphi/2)/w).$$

When, for example, $\varphi = \pi/2$ and $w = 10h$, the number of steps must be $n > 16$. Smoothing the angles φ is realized by taking the requantization line perpendicular to a chord between two points a distance n steps apart on the PWL approximation. The chord may span multiple breakpoints. This procedure is illustrated in Figure 4d.

3.2. Centromere finding

Two new methods for determining the centromere position have been evaluated. The method de-

veloped by Visser (1981), is based on searching the closest pair of opposite contour points. Starting from the thus stretched chromosomes, the straight main axis of the chromosome is known. The head and the tail of the chromosome are deleted. Splitting the chromosome contour into two parts, the size of the deleted parts are chosen such that they match the size of the p terminal of an acrocentric chromosome (otherwise the closest pair of contour points are at the head or the tail). An exhaustive search for the closest pair of points on the clipped contours is performed, resulting in a centromere position. When the two points are at an edge, the chromosome is considered acrocentric. The procedure is illustrated in Figure 6. In the second method of Van Zee (1974), the method is based on the profile of the width of the chromosome, defined as the

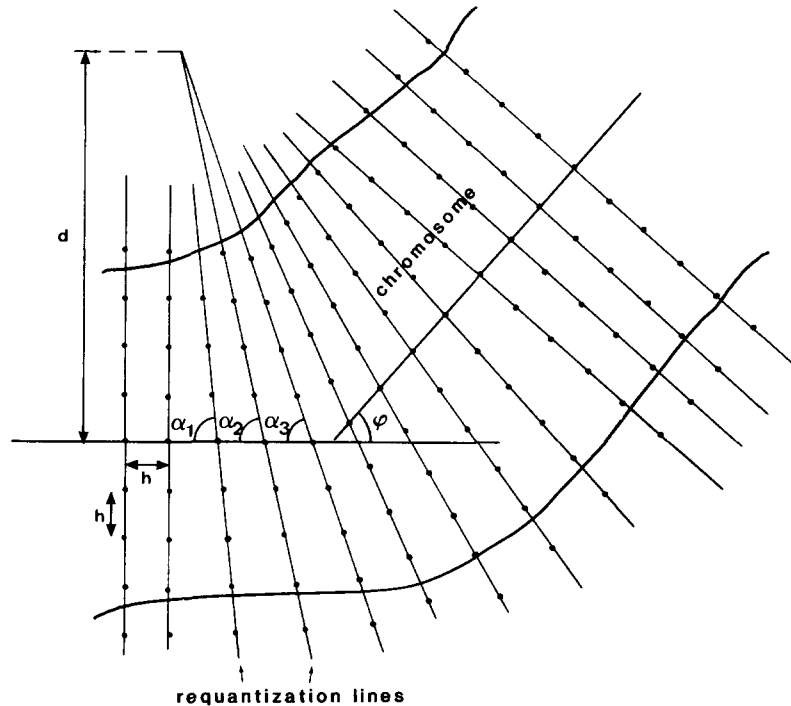


Figure 5. The distance d of the intersection of two successive requantization lines. All intersections are outside the chromosome region. So, $d > w/2$ for all requantization lines, where w is the maximal chromosome width.

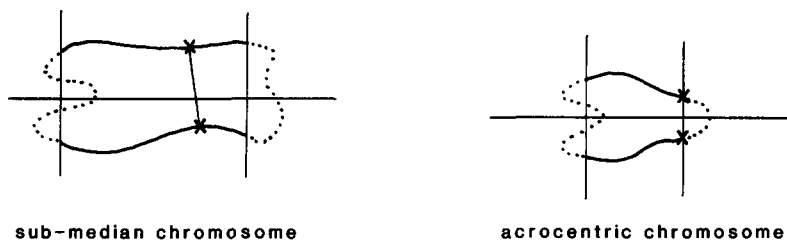


Figure 6. Centromere finding with an exhaustive search for the closest pair of opposite contour points. This pair is found at an edge for an acrocentric chromosome.

distance between the borders measured perpendicular to the main axis. After its construction the profile is smoothed and a relative minimum between two maxima is searched for. When such a minimum exists, a second order polynomial is fitted to the original profile around the minimum, precisely positioning the centromere position. When no relative minimum is found, it is assumed that the chromosome is acrocentric. In that case a third order polynomial is locally fitted to the profile and the minimum of the first derivative (the inflection point) of this polynomial is taken as the centromere position.

The first method gave correct results for 85% of the chromosomes of the Leyden data set and 93% correct results for the Copenhagen data set. The second method gave correct results for 68% of the chromosomes of the Leyden data set and 76% correct results for the Copenhagen data set. A result was counted correct when the distance between the computed centromere location and the manually determined centromere position was less than 10% of the chromosome length, De Muinck Keizer (1984). Results achieved with the first method are satisfactory considering the data set included severely bent chromosomes.

3.3. The Laplace local band descriptor

Bands are only admitted as such in regions where the intensity is sufficiently low. Thus, the chromosome images are first thresholded to locate regions potentially bearing a band. In this way the detection of vague bands and vague connections between clearly separate bands is avoided. The local band descriptor we propose is based on two-dimensional Laplace filtering of the image. This second derivative filtering leads to the detection of hills and valleys (convex and concave regions) in grey value images. In the grey value image, bands form the concave regions and the lighter parts the convex regions. Detection of the bands in this way is in principle insensitive to monotone grey value transforms, Smeulders (1978). The size of the Laplace filter (3×3 , 4×4 or 5×5) is adapted to the size of the band. Because the band descriptor is based upon the two-dimensional image, the bands are also correctly detected in case of unequal local contractions in the chromatids. In this way errors due to the projection of bands are avoided.

The points surviving the thresholding, and also having a positive value after convolution (convex regions), are labeled and every connected set of labeled points is a candidate for a band, as illustrated in Figure 7. Bands, of which the area is below a heuristically set limit (5) are further discarded. When no valid bands are found, the chromosome is rejected from further analysis.

Calculated for each band are:

- (1) Minimum, maximum and middle position by projection of the band perpendicular to the main axis, normalized on the length of the chromosome.
- (2) Area of the band, expressed as the relative chromosome area.
- (3) Darkness of the band, expressed as the grey value sum normalized on the integrated density of the chromosome.

When two bands on separate chromatids coincide, that is when the projected minimum and maximum location are within 2% of the total length, the two bands are merged and the band parameters are recalculated. From the set of band parameters, for each individual chromosome the following features are determined (see Figure 8):

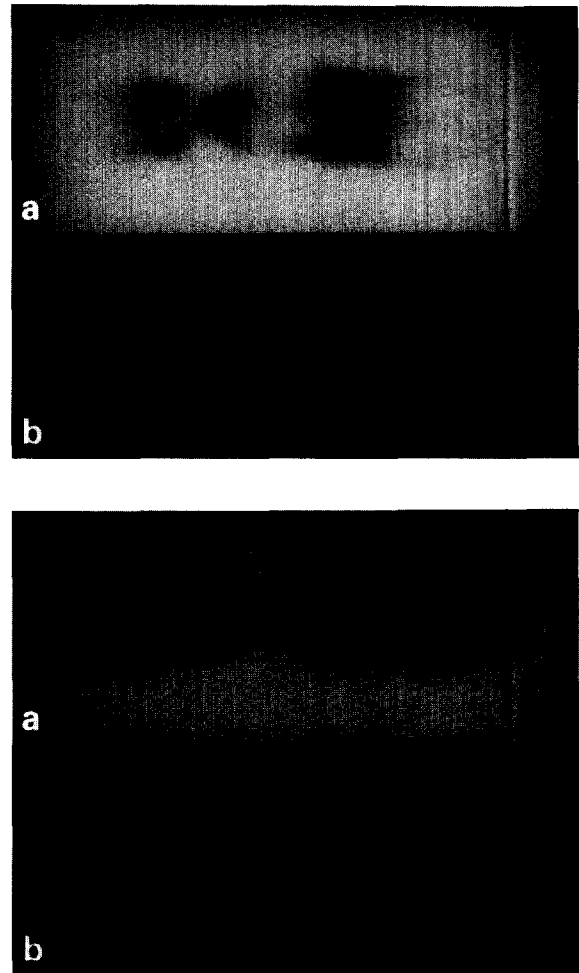


Figure 7. (a) Digitized chromosome, (b) chromosome after filtering.

The location of the band with the largest area on the chromosome.

The location of the darkest band.

The location of the first band after the centromere.

The location of the darkest band on the *p* terminal.

The location of the darkest band on the *q* terminal.

The location of the first band on the *p* terminal.

The location of the last band on the *q* terminal.

From these features the projected middle positions are calculated. Apart from the band information, also the overall length and centromere index are used in the classification.

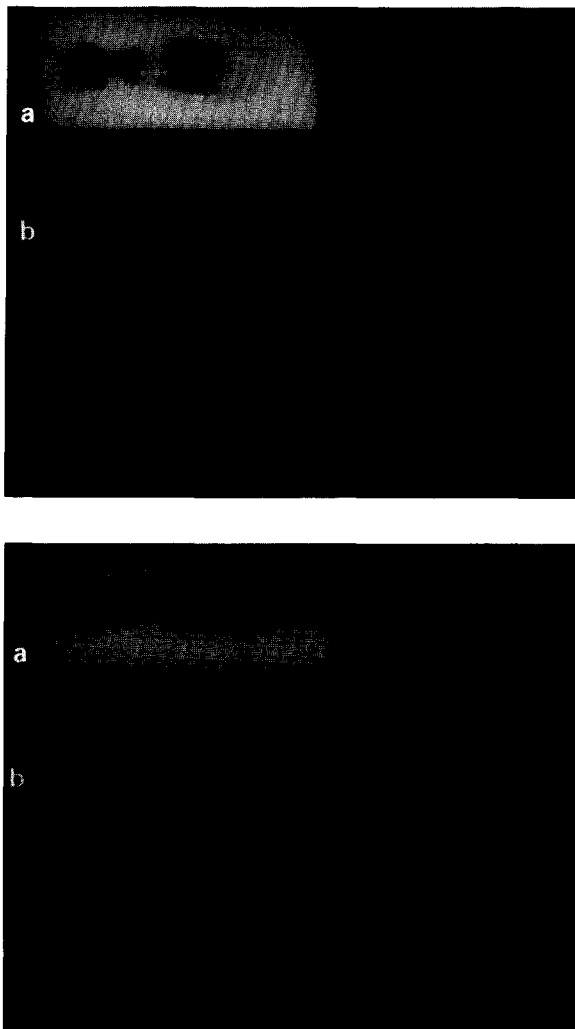


Figure 8. (a) Digitized chromosome, (b) labelled bands.

4. Results

4.1. Data sets used

In this paper we use two data sets, one coming from the Copenhagen group, Lundsteen et al. (1980), consisting of some 7000 chromosomes. The second data set originates from the Leyden group, van der Ploeg et al. (1974), consisting of 920 chromosomes. Both sets contained individual chromosomes, for which the centromere position and class number was given manually.

To demonstrate the potentialities of the local band descriptors, the Copenhagen data set was

used to generate classification results of those chromosome classes which are traditionally difficult to distinguish. They are: the classes 4 and 5, the classes 7 and X and the classes 9 through 12. In a general classification experiment, all 24 classes were used of the Leyden data set. Subsequently, the complete Copenhagen set was processed and used in the ultimate classification experiment.

4.2. Classification procedures

Classification was realized with the ISPAHAN statistical package developed by Gelsema (1981). Several classification methods have been applied: the linear Fisher discriminant, non-parametric Bayes rule and a Nearest Neighbor classification technique, Duda (1973).

The distilled set containing the 1500 chromosomes was used to select the 8 best features using the forward selection procedure.

4.3. Classification results

The classification results are summarized below. Results from the same data set acquired with the WDD features (Granum et al. (1981)) are reported as well for comparison. Best results with the local band descriptors have been achieved with the Bayes rule although NN was nearly as good. The latter, however, is very time consuming. With Fisher discriminant analysis consistently inferior results were obtained. This indicates that either the band description features are far from normally distributed or that there are large differences in the covariance matrices. The method based on the WDD functions gave best results with the Fisher discriminant analysis. Features used were length, CI and the 8 WDD functions.

In Table 1 results are given obtained with the Copenhagen data set for the selected chromosomes. For each classification problem, the data set has been divided in two equal parts: one part is the learning and the other one the test set. In the first column results are given obtained with the Laplace band descriptor, in the second column with our implementation of the WDD functions. The third column gives the results obtained in Copenhagen with the WDD functions. In the Copenhagen exper-

Table 1
Results on the first data set: Copenhagen set, 179 metaphases

DELFT		COPENHAGEN	
1500 chromosomes Different test and learning set Including severely bent chromosomes		6896 chromosomes Test set equals learning set Severely bent chromosomes excluded manually	
Laplace Band Descriptor	WDD Functions	WDD Functions	
Classes	Error rate	Error rate	Error rate
4 and 5	5.4%	5.9%	2.4%
7 and X	1.2%	5.0%	2.1%
9, 10, 11 and 12	9.5%	3.6%	1.8%
	no rejects	no rejects	rejects 0.7%

periment the classification concerned all classes, however bent chromosomes were excluded.

Table 2 renders the results obtained for all 24 chromosome types using the Leyden data set. The first column again shows the result obtained with the local band descriptor, the second one shows the results obtained with the WDD functions in Copenhagen. Because of the relatively small size of the data set per chromosome type the learning and the test set were identical.

Table 2
Results on the second data set: Leyden set, 28 metaphases

DELFT		COPENHAGEN	
28 metaphases 920 chromosomes			
Test set equals learning set All classes		Test set equals learning set All classes	
Laplace Band Descriptor		WDD Functions	
Error rate: 4.0% no rejects		Error rate: 4.1% rejects: 1.6%	

In Table 3 the ultimate results are presented for all 24 chromosome classes using the Copenhagen set. Again the data set has been split up in two equal parts for classification purposes. The set contained about 7300 individual chromosomes, (severely) bent ones included.

5. Discussion

Comparing methods of automated chromosome classification in general is difficult, because of differences in preparational procedures, metaphase selection, pre-processing and criteria for chromosome selection and the possible use of manual interaction, apart from differences in the image processing and classification procedures. In this paper we evaluated two different techniques. In the local band description method projection errors are avoided, but the method is sensitive to the band segmentation. Bands may be split or merged resulting in different first or last bands and changes in their features. This results in multi-modal feature distributions. Because the WDD technique is a global method no band segmentation occurs, but this method depends heavily upon the centromere location and is sensitive to projection errors.

A fair comparison between the two techniques for band pattern description still is a difficult matter.

Table 3
Ultimate results on the first data set: Copenhagen set, 179 metaphases

DELFT		COPENHAGEN	
		179 metaphases 7284 chromosomes	
All classes Different test and learning set 7284 chromosomes		All classes Test set equals learning set 6896 chromosomes Severely bent chromosomes excluded manually	
Laplace Band Descriptor		WDD Functions	
Error rate: 11.5% no rejects		Error rate: 2.1% rejects: 0.1%	

As far as the Copenhagen data set is concerned, when processed with the Delft technique in the first experiment, a relatively small set was used but some notorious difficult classes were classified. Comparison of the first two columns shows: results with classes 4 vs. 5 are about the same. Results with the local band descriptors are better for classes 7 vs. X but worse for classes 9 to 12. Comparison of columns 1 and 2 versus column 3 is difficult because bent chromosomes were left out in column 3. The results are certainly improved by excluding severely bent chromosomes. With regard to the classification of the smaller Leyden data set both methods performed equally well. The value of the experiment is only indicative, however, because the learning set equals the test set. Table 3 shows the overall error rates of both the Laplace band descriptor and the WDD function technique. At first sight the result of 11.5% error rate seems considerable worse compared to the 2.2% error rate obtained with the WDD function technique. Two crucial facts are in favor of the Laplace band descriptor technique however. First, the data set used to evaluate the Laplace technique was not cleaned up, even bent chromosomes were included. Secondly, the results achieved with the WDD function technique are biased towards an optimistic result since the test set was identical to the learning set. Concluding, several useful new techniques for automated chromosome analysis have been described. The first was the piecewise-linear stretching of bent chromosomes, subsequently two algorithms with an high success rate in locating the centromere position have been presented. Finally, the local Laplace band descriptor technique was introduced, results show its potentiality in automated karyotyping.

Acknowledgement

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Ir. Visser and Ir. de Romph laid the basis for the local band description technique.

Appendix: requantization-line angles

Suppose that we divide the angular difference φ between two successive line-parts at a break point equally over n successive requantization lines with angles $\alpha_1, \dots, \alpha_n$ (see Figure 5). Half of these requantization lines will lie on the first line-part and half of them on the second line-part. For reasons of symmetry, the configuration of the requantized lines on one line-part is equivalent to those on the other line-part. The difference arises through the break point between the two requantization lines. Let us first consider two requantization lines with angles α_k and α_{k+1} ($k < n/2$) on one line-part. This is illustrated in Figure 9a. The distance between two successive requantization lines along this line-part is h . We will calculate the distance b_k of the intersection of the two requantization lines to the PWL-axis. From Figure 9 we see that:

$$\text{tg } \alpha_k = b/s, \quad (1)$$

$$\text{tg } \alpha_{k+1} = b/(s+h). \quad (2)$$

As the angular difference φ is divided over n requantization lines, α_k is given by

$$\alpha_k = \pi/2 - k\varphi/n. \quad (3)$$

Combination of (1), (2) and (3) results in

$$b_k = \frac{h \cos(k\varphi/n) \cos((k+1)\varphi/n)}{\sin(\varphi/n)}. \quad (4)$$

The distance b_k , in the region of interest ($k \leq n/2$), is a monotonically decreasing function of k . So the smallest value of b will occur for the highest value of k for which there is no break point in between the two requantization lines.

When there is a break point in between the two requantization lines, we obtain the situation of Figure 9b. The distance h is split into a portion p on the first line-part and a portion $h-p$ on the second line-part. The distance between the requantization lines along the first extended line-part is $x+p$.

When n is odd the break point is in between re-

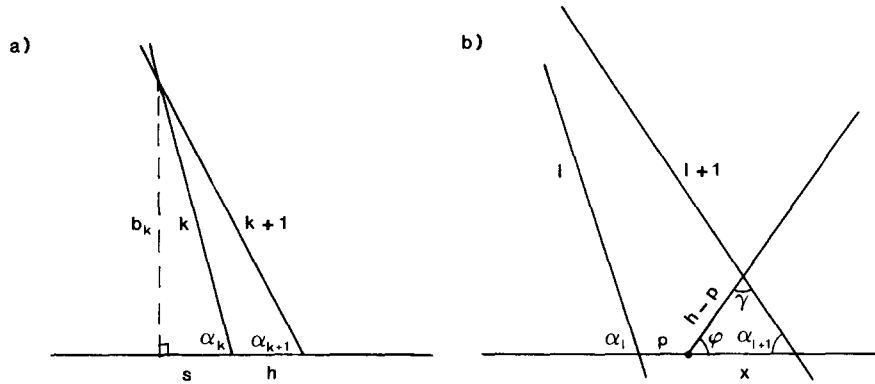


Figure 9. Two different configurations of requantization lines; (a) without a break point in between lines, (b) with a break point in between lines.

quantization line $l = \text{integer}[n/2]$ and $l + 1 = \text{integer}[n/2] + 1$ (Figure 9b). This means that α_{l+1} is smaller than $\pi/2 - \varphi/2$ and as $\gamma = \pi - \varphi - \alpha_{l+1}$, $\gamma > \pi/2 - \varphi/2$ and $\gamma > \alpha_{l+1}$. Thus the line-segment x opposite to γ is larger than the line-segment $h - p$ opposite to α_{l+1} : $x > h - p$ (see Figure 9b).

Two possible pairs of requantization lines may contain the breakpoint when n is even: (a) $l = n/2 - 1$ or (b) $l = n/2$. In situation (a), $\alpha_{l+1} = \gamma = \pi/2 - \varphi/2$ and so $x = h - p$. In situation (b), $\gamma > \alpha_{l+1}$ and $x > h - p$. When there is a break point in between the requantization lines the distance between them along the extended line-part ($x + p$) is always larger than or equal to h . This means that b_l will be greater or equal to the value obtained in the case that there was no break point in between the requantization lines. Thus, the value obtained, when the highest value of k ($n/2$) is taken and b_k is calculated as if there were no break point between the two requantization lines, will always be smaller than the distance of an intersection to the PWL-axis.

As an intersection must lie outside the chromosome region to prevent requantization errors, this condition is fulfilled if we choose

$$b_{n/2} > w/2.$$

Approximating $b_{n/2}$ by

$$h \cos^2(\varphi/2)/\sin(\varphi/n)$$

results in

$$n > \varphi/\sin^{-1}(2h \cos^2(\varphi/2)/w).$$

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