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A software tool to straighten curved chromosome images

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Abstract

A software tool for straightening curved chromosomes has been developed and integrated into the freely available image analysis application *Image SXM* (available via the Internet at http://reg.ssci.liv.ac.uk). This new tool straightens curvilinear objects in one simple step after minimal input from the user. The ends of a curvilinear chromosome are identified by the user using the mouse and a window is opened displaying the object as it would appear if it was straightened out. This image processing produces linear images of chromosomes with no loss of resolution or spatial calibration, making subsequent analysis significantly more straightforward.

Introduction

The use of long or extended chromosome analysis remains a very powerful cytogenetic tool and has been widely applied in the study of human, animal and plant chromosomes. Several evident longitudinal differentiations by banding, structural component types, or by natural colour density differences, have been generating a large amount of cytogenetic information in evolutive, systematic and medical research (Sumner 1990, Verma & Babu, 1995). Among these differentiations, the constrictions, heterochromatic regions, band patterns and chromomeres are best suited for use as cytogenetic markers if they are analysed by high-resolution techniques or in an elongated chromosome form (Spowart 1994, Bickmore & Craig 1997). It is a well-known fact that the long pachytene and polytene chromosomes are particularly advantageous in precise delineation of chromomeres and bands, for the characterization and identification of the specific chromosomes, or for studying structure/function relationships (Sybenga 1992, Kress 1996). However, the longer the chromosomes are, the more sinuous they can be, and detailed scrutiny becomes a problem and can be very tedious (Sumner 1990).

In this paper, we describe a software tool that has been developed to circumvent these problems associated with the analysis of curvilinear chromosomes. An image processing routine has been integrated into the Macintosh software package *Image SXM* (Barrett 2002) that will straighten the image of a curvilinear object such as a chromosome. As the straightened image has the same resolution and spatial calibration as the original image, the variation of intensity and hence the banding pattern along the length of the

chromosome can be readily obtained and compared with other images. Overlapping chromosomes, including self-overlapping or 'knotted' chromosomes, can be handled by the routine provided the overlapping regions do not represent a large proportion of the total chromosome length.

There are commercial software packages that offer very comprehensive chromosome analytical tools (see Carothers & Piper 1994 for a review of computer analysis of human chromosomes). While such software has existed for many years, it has remained in the realm of clinical cytogenetics, where accurate identification of chromosome bands and location of fluorescent probes for banding has received much attention. Unfortunately, software of this sophistication is expensive and has been created specifically for human and only a few other mammalian karyotypes.

Some non-commercial image analysis software packages also provide the ability to straighten the images of curvilinear objects. One such package is Image/J (Rasband 2002) for which a software plug-in module exists (Kocsis 2000). To use this tool, the user selects a set of nodes using the mouse and a non-uniform cubic spline is carried out to define the centreline of the object.

The advantage of the software tool described here is simplicity, as the user need only identify the two ends of the object and the centreline is then determined automatically. If many curvilinear chromosomes require analysis, then this simple and user-friendly approach is very desirable to maximise efficiency and throughput.

Materials and methods

The four long and curved chromosomes chosen as examples for straightening were as follows:

Human high-resolution chromosomes

The high-resolution (550 level bands) mitotic G-banding chromosome number 1 was obtained by the ethidium bromide synchronization technique. Human peripheral blood (0.2 ml) was cultured in 5 ml RPMI 1640 (Sigma), supplemented with 20% fetal calf serum and containing 100 µl phytohaemaglutinin (Sigma) and incubated at 37°C. After 70 h of incubation, 30 µl 1-mg/ml ethidium bromide (Sigma) was added for 70 min, 50 µl 1-µg/ml colcemid (Sigma) was added for 30 min and the culture was incubated at the same temperature. Cells were immediately centrifuged at 100*g* for 10 min, resuspended in 0.075 mol/L KCl, and incubated for 20 min at 37°C. The supernatant was aspirated off and 10 ml fresh 3:1 methanol: acetic acid fixative were added. The fixative was changed three times at 15-min intervals. The GTG-bands (G-bands by trypsin using Giemsa) were obtained using a standard trypsin technique (Seabright 1971).

Chironomus polytene chromosome

Chironomid polytene chromosome number 2 was obtained by a conventional 0.5% lactic–orcein technique (MacGregor 1988).

Maize HKG pachytene chromosome

HKG banding pachytene chromosome number 3 was obtained by the methodology described by Carvalho & Saraiva (1997) and Teixeira & Carvalho (2000).

Maize HKG high-resolution chromosome

High-resolution mitotic HKG-banding chromosome number 5 was obtained as described by Carvalho & Saraiva (1997).

Image analysis

Figures of chromosomes were captured with a CCD video camera on an Olympus BX 60 microscope with a 100× objective lens. The video image was digitized by an Optronics DEI-470 image analysis system attached to a Power Macintosh G3 computer. Image analysis was performed on a Power Macintosh G4 computer using the freely available *Image SXM* software (Barrett, 2002) which can be obtained via the Internet at http://reg.ssci.liv.ac.uk. This is a spin-off of the public domain image analysis application *NIH Image* which was developed by Rasband (1998). The original colour TIFF chromosome images were converted to a 256 grey value and the scale was standardized to the range from 0 (white) to 255 (black). The density plots were performed using the plot profile feature of the software.

The tool to straighten curved chromosomes

The algorithm used to straighten curved chromosome images comprises five steps, shown schematically in Figure 1.

Step 1
The locus of the centreline of the chromosome is determined by a routine that moves along the chromosome a few pixels at a time. At each point, the intensities of neighbouring pixels of the image on the circumference of a circle of a few pixels radius are measured. These intensities are used to determine the local width of the chromosome and the position of the next point of the estimated centreline. This process starts from the point identified by the user as the start of the chromosome, and continues until the end of the chromosome is reached. The radius of the circle is varied automatically along the chromosome such that it remains slightly larger than the local width of the chromosome. This ensures that the routine can cope with the rapid changes in direction that can occur if the chromosome is tightly curved.

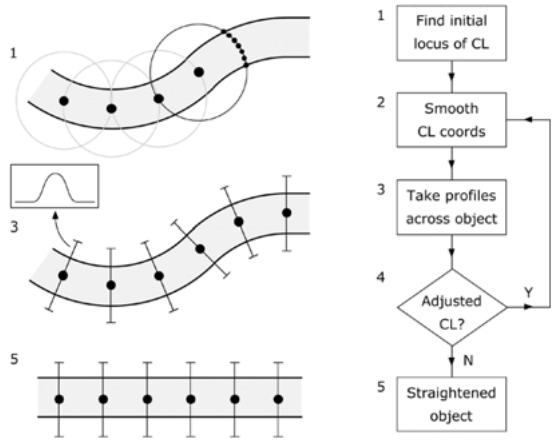

Figure 1. Schematic representation of the five steps used to straighten curved chromosome images. The flow diagram on the right shows how the algorithm loops until a satisfactory result has been achieved (CL refers to the centreline of the chromosome). Steps 1, 3 and 5 are illustrated on the left, where the large points are on (or close to) the centreline of the chromosome. The inset above step 3 shows one of the many intensity profiles that are taken along the chromosome.

Step 2
The locus of the centreline is then smoothed using a customized smoothing function. The relative weightings of neighbouring points of the locus used in the smoothing function were chosen judiciously to meet two criteria: (1) to reduce small-scale fluctuations in the locus of the centreline, resulting from noise in the original image and small irregularities and asymmetries in the width of the chromosome; (2) to preserve local quadratic curvature of the locus so that tight curves are not rounded excessively.

Step 3
Intensity profiles of the chromosome are taken at each point, with the direction of the profile set perpendicular to the local tangent of the locus. If a point is located on the true centreline of the chromosome, then such a profile will be symmetrical about its midpoint.

Step 4
Each point on the locus is moved by a small displacement to make the profile more symmetrical. Steps 2–4 are repeated until there is no further reduction in the mean displacement. Typically, after two or three iterations, the mean displacement of all the points in the locus will be no more than a few tenths of a pixel.

Step 5
When the locus has been optimised, interpolating between the points generates a new locus in which the points are separated by one pixel. Intensity profiles are calculated as described in Step 3 and used to generate the straightened image. Finally, the length of the straightened chromosome is calculated.

For an image comprising 100 000 pixels, this algorithm will be executed in a few tenths of a second on a computer with a processor clock speed of 1 GHz. Thus there is the potential to process automatically an image comprising many chromosomes in only a few seconds. The algorithm has been written so that it is not particularly sensitive to the level of contrast of the chromosome image and is equally effective on bright-field or dark-field images.

Figure 2. High-resolution HKG-banding mitotic maize chromosome before (**a**) and after (**b**) application of the straightening tool. Bar = 5 μm.

Figure 4. Maize pachytene chromosome Giemsa stained showing (**a**) original shape, (**b**) the same shape after line selection and (**c**) the straightened chromosome. Bar = 5 μm.

Results and discussion

Cytogenetic preparations produced long and isolated chromosomes from root tips (Figure 2) and microspore cells (Figure 4) of maize, in polytenic form from *Chironomus* (Figure 5), and in a G-band high-resolution chromosome pattern from human lymphocyte culture (Figure 6). All chromosomes showed good cytogenetic quality, adequate longitudinal banding resolution and curved morphology that was appropriate for tests of the straightening tool.

The routine to straighten curvilinear chromosomes was tested in manual mode on the HKG-banding metaphase maize chromosome number 5. This chromosome shows large heterochromatic blocks as morphological marks at both arms (Figure 2a). By selecting a freehand line icon and tracing over the chromosome from one end to the other, and choosing the straightening tool in

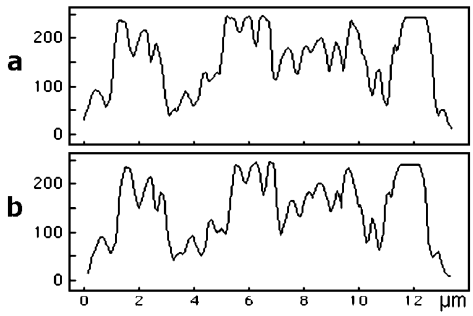

Figure 3. Comparison of density profile of the grey-scale value for maize chromosome from Figure 2, before (**a**) and after (**b**) application of the straightening tool. It can be observed that there is hardly any difference between the two plots.

Figure 5. Polytene chromosome of *Chironomus*, showing (**a**) its original shape and (**b**) its straightened shape. Notice that the band pattern remains the same after application of the straightening tool. Bar = 20 μm.

Figure 6. G-banding human chromosome before (**a**) and after (**b**) application of the straightening tool. Bar = 5 μm.

the option menu, a linear chromosome image was generated (Figure 2b) without loss of position or density, as can be seen in the plot profile (Figure 3a, b).

The pachytene maize chromosome was a good test to demonstrate the use of the straightening tool since its morphology is long and sinuous in most cytological preparations and it has a typical chromomere pattern. Using the double-mouse-click mode, on the start and the end points of chromosome number 3, shown in Figure 4a, the straightening tool automatically traced out the centreline selection (Figure 4b) of the chromosome, and straightened it in a near-perfect linear shape which respected the chromomere pattern (Figure 4c).

Another example of the straightening tool application in cytogenetic analysis was verified on the polytene chromosome of *Chironomus* and high-resolution bands in human chromosomes. Using the tool for a polytene chromosome image, which shows a pattern of many dark and light bands side by side (Figure 5a), resulted in a straightened form with all longitudinal bands displayed in positions corresponding to those of the original (Figure 5b). Similarly, the high-resolution human chromosome sample (Figure 6a) was also straightened (Figure 6b), and the results demonstrate that the procedure simplifies band-pattern recognition.

The development and inclusion of the straightening tool in the *Image SXM* software has increased the possibilities of analysing chromosome morphology. This application, in its ability to straighten plant, animal and human chromosomes, will find use in a diversity of cytogenetic approaches, such as karyotype and idiogram assemblages, banding pattern analysis, chromomere mapping, probe positioning, morphological comparisons and structural variations.

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