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Automation of Cytogenetics

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Contents

Chromosome Aberration Detection with Hybridized DNA Probes: Digital Image Analysis and Slit Scan Flow Cytometry

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Summary

Metaphase chromosomes of Chinese hamster × human hybrid cell lines were hybridized with biotin-labelled human total genomic DNA. The hybridized DNA and hence the human chromosomal material was visualized using either an alkaline phosphatase enzyme reaction or a FITC labelling detection system. For quantitative evaluation, both digital image analysis and slit scan flow cytometry were applied. The results suggest that the automatic detection of chromosome translocations with hybridized DNA probes is feasible both on slides and in suspension.

1. Introduction

An important field of automated cytogenetics is the detection of structural chromosome aberrations. While considerable progress has been made concerning the automated evaluation of dicentric chromosomes from homogeneously stained specimen (Gray and Langlois, 1986; Lörch and Stephan, 1986), the automated detection of other structural aberrations, such as translocations, is still in the beginning (Piper and Lundsteen, 1987; Willborn et al., 1987).

Here, an alternative to the "conventional" method based on the analysis of banded chromosomes is presented. The rationale of this new approach is the use of hybridization techniques to visualize specific chromosomes and chromosome subregions (Durnam et al., 1985; Manuelidis et al., 1985; Schardin et al., 1985; Cremer et al., 1986, 1988a; Pinkel et al., 1986; Emmerich et al., 1989). To exemplify the method, chromosomes from Chinese hamster × human hybrid cell lines are used. It is anticipated, however, that recent progress made in the visualization of specific chromosomes in human cells by in situ suppression hybridization using recombinant DNA libraries from sorted human chromosomes (Cremer et al., 1988b; Lichter et al., 1988) will make it feasible to use the approach, described here, also for the automated detection of chromosome rearrangements, such as translocations, in human cells.

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2. Materials and Methods

For the evaluation of metaphase spreads, the Chinese hamster × human hybrid cell line GB3 (kindly provided by C.H.C.M. Buys, Groningen), containing 18 hamster chromosomes and two No. 13 chromosome equivalents as the only human material, was grown under standard conditions. Mitotic cells were enriched with demecolcine (Colcemid; 0.25μg/ml medium) and fixed on slides with methanol/acetic acid (3:1). The metaphase spreads were hybridized with biotinylated human total genomic DNA and the hybridized DNA was visualized with the alkaline phosphatase (AP) method essentially as described by Schardin et al. (1985) and Cremer et al. (1986).

For the analysis of isolated chromosomes in suspension, the Chinese hamster × human hybrid cell line A_1wbf_2 (kindly provided by P.Pearson, Leiden) was used, containing four human chromosomes and several interspecies translocations. The chromosomes were hybridized in suspension with biotinylated human total genomic DNA as described (Dudin et al., 1987a). Human material was detected due to the binding of FITC labelled antibodies (goat anti rabbit - rabbit anti biotin) or a streptavidin - FITC complex to the biotinylated hybridized DNA (Dudin et al., 1988; Hausmann et al., 1989).

In both cases the chromosomes were counterstained with propidium iodide (PI). Counterstaining with DAPI is also possible (Dudin et al., 1987b, Hausmann et al., 1988).

Digital image analysis of colour microphotographs of chromosomes on slides was performed using a Joyce-Loebl drum scanning densitometer (Scandig 2605) and a VAX 11/780 as described (Dudin et al., 1988, Hausmann et al., 1989).

Two parameter slit scan flow cytometry of isolated chromosomes in suspension was done on the Amsterdam slit scan flow cytometer (Aten et al., 1988, Hausmann, 1988). Briefly, the chromosomes pass a focussed laser beam (ca. 3μm diameter). The time dependent fluorescence signal is registered and thus produces a profile of the fluorescence distribution along the chromosome (Gray et al., 1979; Lucas et al., 1983; Lucas and Gray, 1987).

The system used is based on an Ortho Cytofluorograph. Simultaneous PI and FITC excitation were performed with an argon laser at 488nm with 500mW. The PI fluorescence was collected on channel 1 while the FITC emission was collected on channel 2 via a 525nm band pass filter.

3. Results

Figure 1 shows a metaphase spread of the Chinese hamster × human hybrid cell line GB3 following hybridization, alkaline phosphatase (AP) visualization of hybridized chromosome sites, and PI counterstaining. In this cell an interspecies translocation including a part of a human (black AP staining) and a Chinese hamster chromosome (PI staining) is clearly visible.

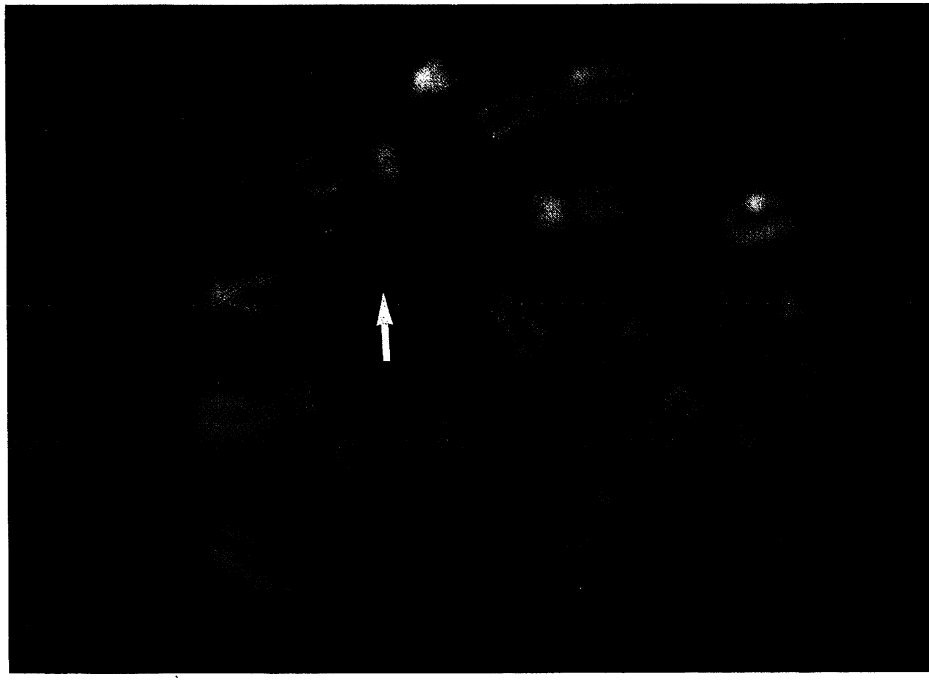

Fig. 1. Metaphase spread of a cell of the Chinese hamster × human hybrid cell line GB3 following in situ hybridization with biotinylated human total genomic DNA. The hybridized site indicating human material (arrow) is visualized by an alkaline phosphatase enzyme reaction (black staining).

Figure 2 shows the integrated density profiles of the chromosomes of the metaphase spread of figure 1. All Chinese hamster chromosomes have either a "bimodal" (e.g. figure 2a-e) or a "smooth" (e.g. figure 2 m-n) distribution as expected for homogeneous staining. The only "aberrant" profile observed is that of the interspecies translocation chromosome (figure 2j). In this case, two maxima of considerably uneven heights were observed. Thus, the translocation chromosome may be distinguished by quantitative characteristics. For example, the contrast

$$c = \frac{|\text{maximum } 1 - \text{maximum } 2|}{\text{maximum } 1 + \text{maximum } 2}$$

calculated for bimodal profiles of normal chromosomes was between 0.03 and 0.16 while for the profile of the translocation chromosome (figure 2j) a c-value of 0.36 was obtained. In case of a very strong AP reaction, PI fluorescence on the human part of the translocation chromosome may be suppressed completely. This may result in a "smooth" profile of the fluorescence detectable along the translocation chromosome. Additional information to distinguish such a case from chinese hamster chromosomes with a "smooth" fluorescent profile can

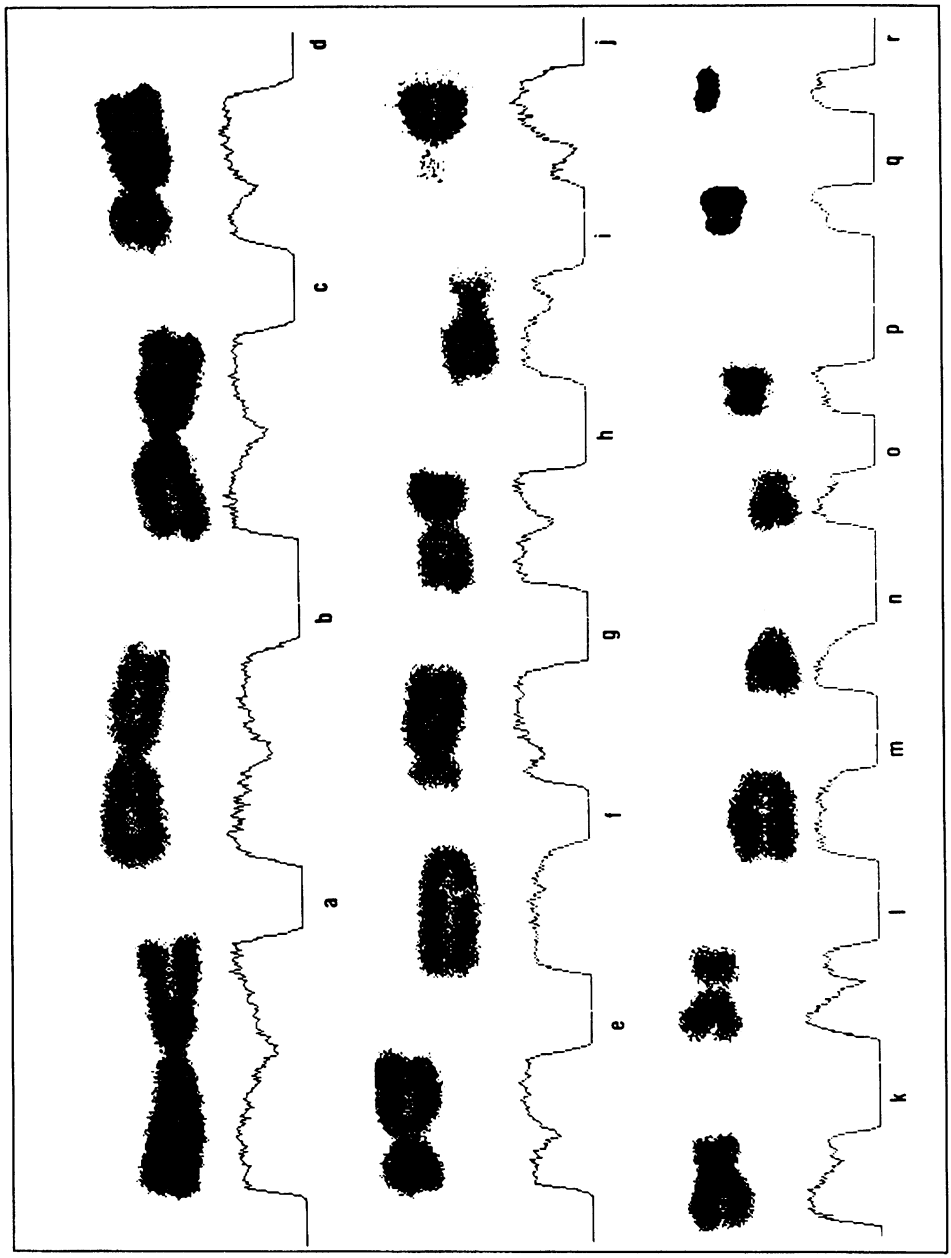

Fig. 2. Integrated density profiles (sum of gray level values perpendicular to the chromosome axis) of the chromosomes of figure 1. Chromosomes were numbered (a - r) according to length. For all chromosomes with "bimodal" profiles (a - i and k - l) the maximum of the profile on one arm is less than 1.4 higher than on the other. On profile j, however, the intensity on the long arm (Chinese hamster material) is 2.3 times higher than on the short arm (alkaline phosphatase labelled human part of the chromosome).

easily be obtained by digital image analysis of microphotographs taken with transmittant light which allows the direct measurement of accumulating dye due to the AP reaction.

In many cases translocation chromosomes are rare events. Therefore it may be important to accelerate the classification of chromosomes according to "normal" and significant "aberrant" profiles. Slit scan flow cytometry allows to acquire profiles of stain distribution along chromosomes (Lucas et al., 1987) at a rate up to 100/sec. Classification of the chromosomes is possible for instance according to the centromeric index (CI) of the fluorescence intensity profile. Therefore, it appeared to be interesting to examine whether this "one dimensional imaging" technique may be combined with the hybridization approach using a fluorochrome (FITC) detection system instead of AP reaction.

The morphology of chromosomes following fluorescence hybridization in suspension was well preserved (see also Dudin et al., 1987a). Integrated density profiles obtained for randomly selected FITC and/or PI stained chromosomes in suspension from several Chinese hamster × human hybrid cell lines were very similar to profiles obtained from conventional metaphase spreads (see also Hausmann et al., 1989). The rather homogeneous FITC staining pattern indicates a correspondingly homogeneous hybridization of human DNA sequences along the arms of isolated human chromosomes. Analogous results were obtained for the PI distributions along the Chinese hamster chromosomes of these cell lines.

For quantitative evaluation of CIs from integrated density profiles, different ways of calculation are possible (figure 3):

$$\text{(a) CI(L)} = \frac{\text{length of long arm}}{\text{entire chromosome length}},$$

$$\text{(b) CI(A)} = \frac{\text{area of long arm profile}}{\text{entire profile area}}$$

After staining with a DNA specific fluorochrome the entire profile area should be directly proportional to DNA content (and to chromosome length). Accordingly, there should not be any great variation in the linearity between CI(L) and CI(A).

Using these two modes of evaluation, CIs were obtained from chromosomes of the A_1wbf_2 cell line. As expected, in the case of a homogeneous binding of PI to the DNA of Chinese hamster chromosomes a linear relationship exists between CI(A) and CI(L) (figure 4b). The same linear relationship is also expected in the case of a homogeneous binding of the biotinylated human DNA to human chromosomes detected with FITC-streptavidin or a FITC labelled double antibody system. Figure 4a shows that this is indeed the case with high accuracy.

It is concluded that under the conditions used, a homogeneous FITC distribution on both chromosome arms corresponding to CI(A) being proportional to CI(L) will denote a normal chromosome. Alternatively, a non-homogeneous distribution of hybridized biotinylated human DNA, i.e. a strong

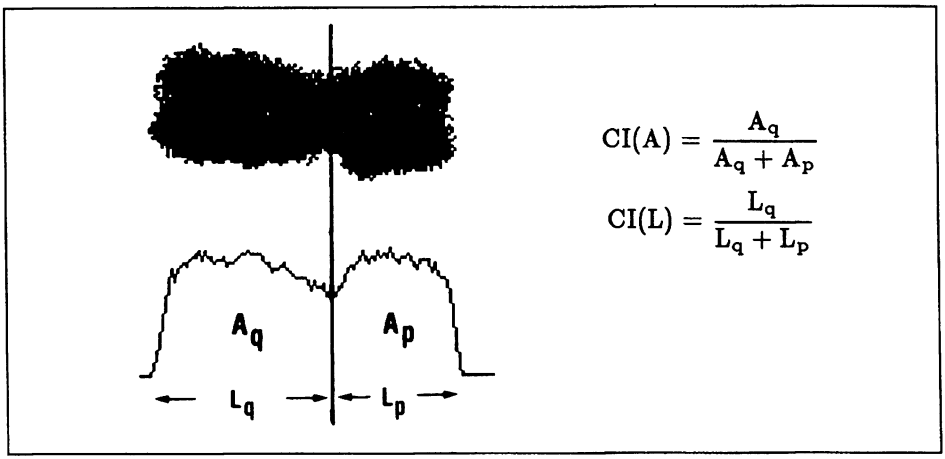

Fig. 3. Determination of the centromeric index (CI) from integrated density profiles (see text).

non-homogeneous FITC distribution, along the arms of a given target chromosome suggests a chromosome consisting of both Chinese hamster and human DNA (interspecies translocation).

Using two parameter slit scan flow cytometry, profiles of chromosomes (A_1wbf_2 cell line) were measured following fluorescence hybridization in suspension, FITC labelling, and counterstaining with PI (Hausmann et al., 1989). For every chromosome, FITC and PI fluorescence was measured simultaneously. In this case, the PI fluorescence distribution was used only to assure that the chromosome alignment was correct (i.e. ideally, in case of metacentric and submetacentric chromosomes a bimodal distribution is expected).

Figure 5 shows the PI-FITC dual parameter slit scan profiles of a presumably normal (a) and a presumably aberrant chromosome (b). The bimodal PI distribution indicates that the flow alignment was correct. In case (a), the symmetrical FITC distribution with two peaks indicates a profile of a "normal" chromosome; in case (b), however, the FITC profile is significantly asymmetrical. Only one FITC peak is clearly distinguishable in channel 2 and corresponds to the left PI peak in channel 1. A careful examination (Hausmann et al., 1989) of the slit scan profiles of about a hundred of correctly aligned chromosomes in combination with a microscopic evaluation led to the conclusion that aberrant profiles of this type are indeed due to structural chromosome aberrations (in this case an interspecies translocation).

4. Discussion

Recently, it has been shown (Durnam et al., 1985; Schardin et al., 1985; Pinkel et al., 1986) that interspecies translocations in metaphase spreads of Chinese

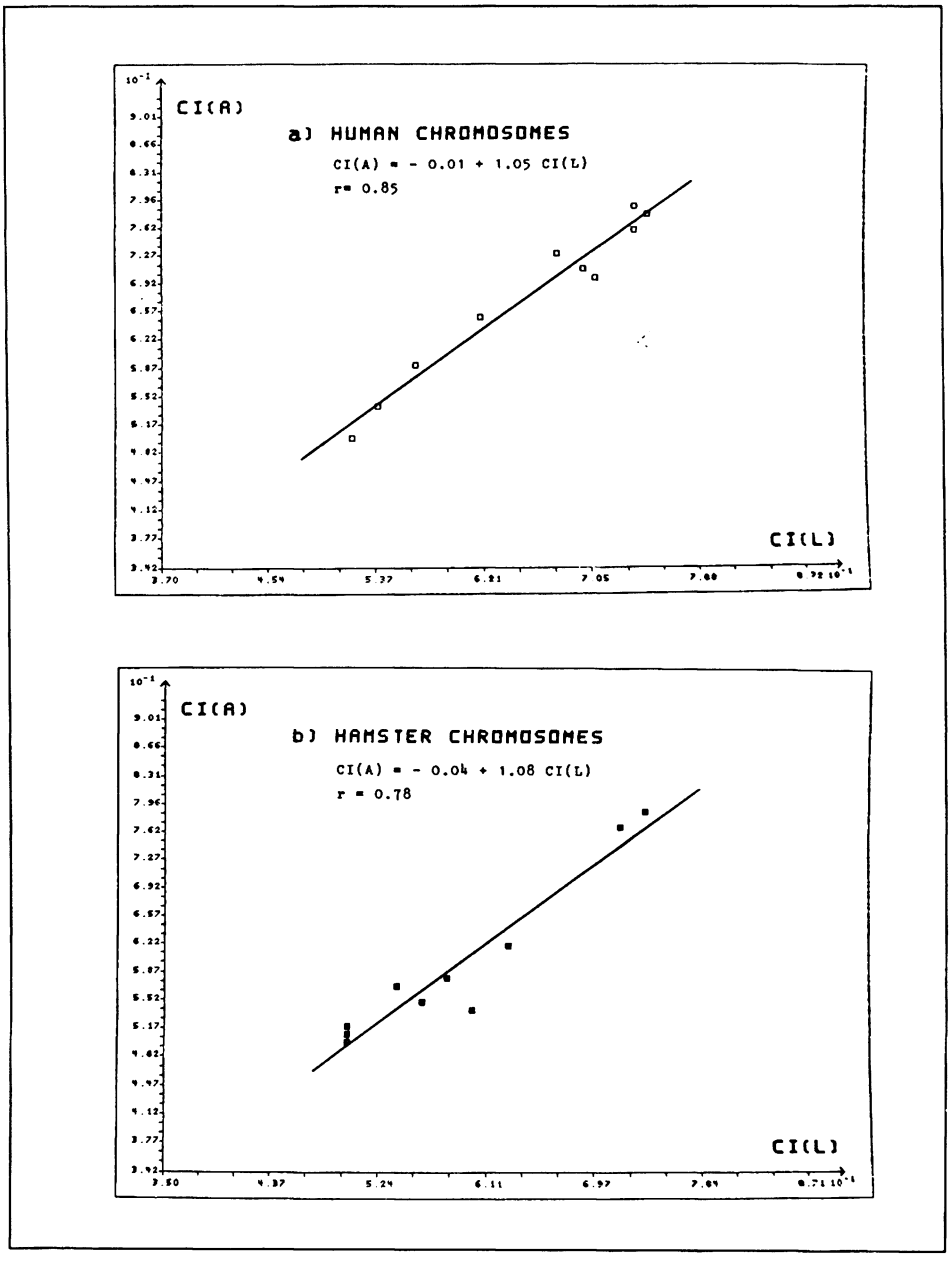

Fig. 4. Ordinate: CI(A): Centromeric index as determined from density profile areas (see figure 3 and text) Abscissa: CI(L): Centromeric index as determined from profile length (see figure 3 and text) a) Relationship between CI(A) and CI(L) for FITC fluorescing chromosomes in suspension (human chromosomes) b) Relationship between CI(A) and CI(L) for PI fluorescing chromosomes in suspension (chinese hamster chromosomes)

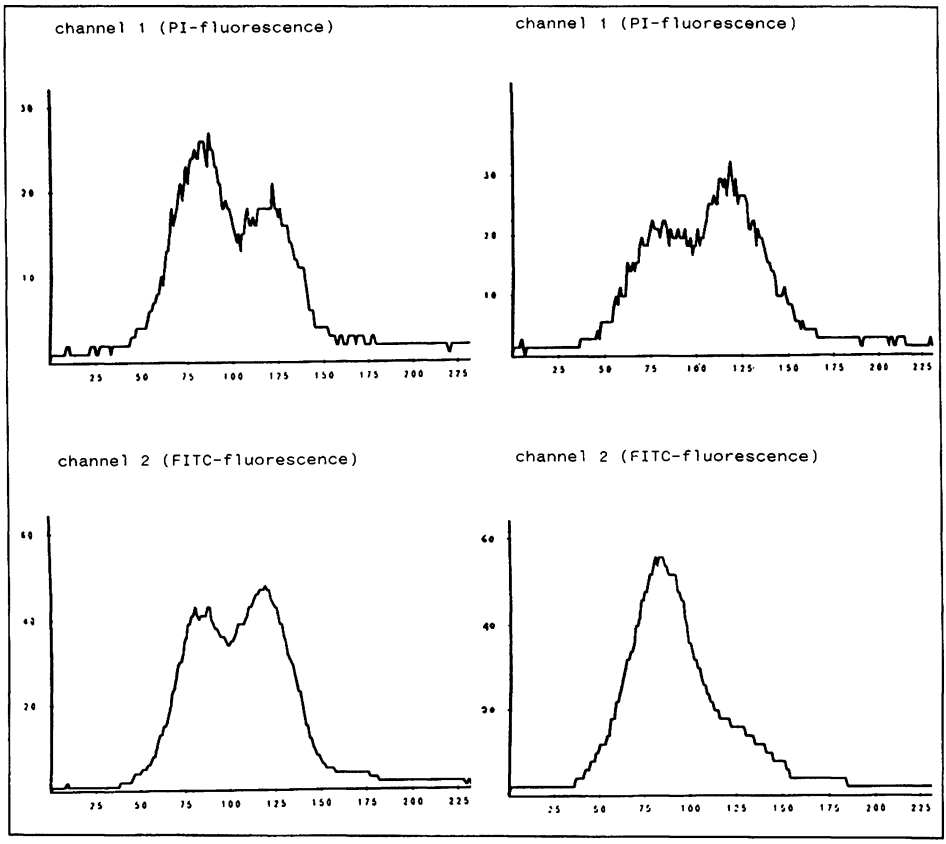

Fig. 5. Dual parameter slit scan profiles of (left) a presumably normal chromosome, (right) a presumably aberrant chromosome. Ordinate: Fluorescence intensity (channel 1: PI fluorescence, channel 2: FITC fluorescence). Abscissa: Time of flight (in units of 100nsec)

hamster × human hybrid cells may easily be assessed by microscopic evaluation following in situ hybridization with biotinylated human genomic DNA. No image analysis, however, was performed in these studies.

The results presented here suggest that automated detection of translocations following hybridization with specific DNA probes is feasible. This is shown both by conventional digital image analysis of microphotographs of metaphase spreads hybridized on slides and by two parameter slit scan flow cytometry following fluorescence hybridization of isolated chromosomes in suspension. So far, the method has been used for interspecies translocations. These are by themselves interesting, e.g. for dosimetry of radiation induced aberrations (Pinkel et al., 1986). Recently, however, chromosome specific DNA libraries (Van Dilla et al., 1986) were used to visualize specifically individual metaphase chromosomes in human normal and aberrant cells by in situ suppression hybridization techniques (Cremer et al., 1988b; Lichter et al., 1988).

This will allow to extend the approach described here to the automated detection of structural chromosome aberrations in human cells, e.g. for monitoring of radiation damage, or application in constitutional and tumor cytogenetics.

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