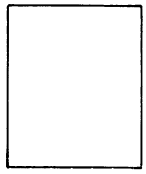

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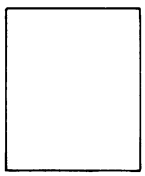

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PROGRAM AND PROCEEDINGS

F. Persillon

CONTENTS

non-isotopic in situ hybridization and digital image analysis of chromosomes in mitotic and interphase cells

T. CREMER, B. REMM**, I. KHARBOUSH*, A. JAUCH*, J. WIENBERG*, E. STELZER***, C. CREMER**, *****

Visualization of individual chromosomes, chromosomal subregions and genes has become possible at all stages of the cell cycle by recent advances in non-isotopic in situ hybridization. In combination with two-and three-dimensional digital microscopy and automated procedures of image analysis this approach can help to reduce the human workload and open new avenues in clinical cytogenetics, as well as in studies of the three-dimensional chromosome organization in the cell nucleus. Examples are presented for the automated image analysis of chromosome translocations, as well as for the analysis of the dystrophin gene for specific intragenic deletions. Further examples illustrate the potential to study the orientation, size and shape of individual interphase chromosome domains in combination with confocal fluorescence microscopy. In an outlook, we discuss strategies and problems to improve the versatility and sensitivity of this approach by use of multicolor in situ hybridization protocols in combination with digital image analysis.

DEMAND FOR NEW APPROACHES TO OVERCOME THE LIMITATIONS OF CONVENTIONAL CYTOGENETICS

Conventional cytogenetics — in spite of all its important results since the introduction of the first chromosome banding method by Caspersson, Zech and their colleagues more than twenty years ago (Caspersson et al. 1968) — has suffered from several limitations : 1. The resolving power of chromosome banding techniques has been limited to the megabase (Mb) range of DNA, even when high resolution banding is applied. The resolution, which can be routinely obtained, is often further impaired due to the lack of chromosome preparations with long, optimally spread and banded chromosomes. 2. The analysis of banded chromosomes is time consuming and requires skilled personnel. Efforts of automatization have met with limited succes so far, in particular in the case of tumor cells with multiple structural aberrations. 3. The nucleus of proliferating and of terminally differentiated cells has been largely off-limits to the cytogeneticists. Until recently cytogeneticists have lacked the methods to answer basic questions as to the size, shape, number and specific arrangements of chromosomes in the nucleus of normal and aberrant cells. The fraction of mitotic cells available for chromosome analysis, may not only be small but also far from representative for the cell population as a whole. For example one may consider the heterogeneous mixture of tumor cell subclones contained in many solid tumors with their different biological propensities, cell cycles, growth requirements and distinct differences of chromosome aberration patterns (see for example Fearon and Vogelstein 1990). Some of these cells may be selectively propagated during in vitro cultivation, while others are lost.

CHROMOSOME PAINTING BY NON-ISOTOPIC IN SITU HYBRIDIZATION

Recent progress in non-isotopic in situ hybridization (for review see Raap et al. 1990 ; Lichter et al. 1991) digital microscopy and image analysis (Agard et al. 1989 ; Jovin and Arndt-Jovin 1989 ; Lundsteen and Piper 1989) has provided new tools to overcome the limitations mentioned above. In situ suppression (CISS-) hybridization protocols have been designed to suppress the signals of interspersed repetitive sequence elements which in addition to site specific sequences are present in many cloned DNA probes (Landegent et al. 1987 ; Cremer et al. 1988a, Lichter et al. 1988a, Pinkel et al. 1988). By use of appropriate DNA-probes, such as bacteriophage or plasmid DNA libraries from whole human chromosomes and chromosomal subregions, as well as individual cosmid- or YAC-clones, selective staining of individual chromosomes and chromosomal subregions has become possible, down to the level of individual genes. CISS-hybridization has already become an important adjunct in cytogenetics, including pre- and postnatal chromosome diagnostics (Pinkel et al. 1988 ; Lichter et al. 1990 ; Jauch et al. 1990), tumor cytogenetics (Cremer et al. 1988a), biological dosimetry (Cremer et al. 1990 ; Popp et al. 1990a), chromosome evolution (Wienberg et al. 1990), and gene mapping (for review see Lichter et al. 1991). In the course of these developments cytogeneticists have become able to pinpoint chromosome aberrations with unprecedented resolution at all stages of the cell cycle, even in terminally differentiated cells (Kolluri et al. 1990 ; Anastasi et al. 1990), in a way which is particularly suitable for the requirements of digital image analysis.

In the following we will apply the term chromosome painting (first introduced by

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We thank our colleagues Marion Cremer, Vera Malher and Susanne Popp for providing the in situ hybridization experiments shown in Fig. 2a, b and 3a, and Angelika Wiegenstein for excellent photographic work. This work was supported by the Deutsche Forschungsgemeinschaft (Cr 59/11-1).

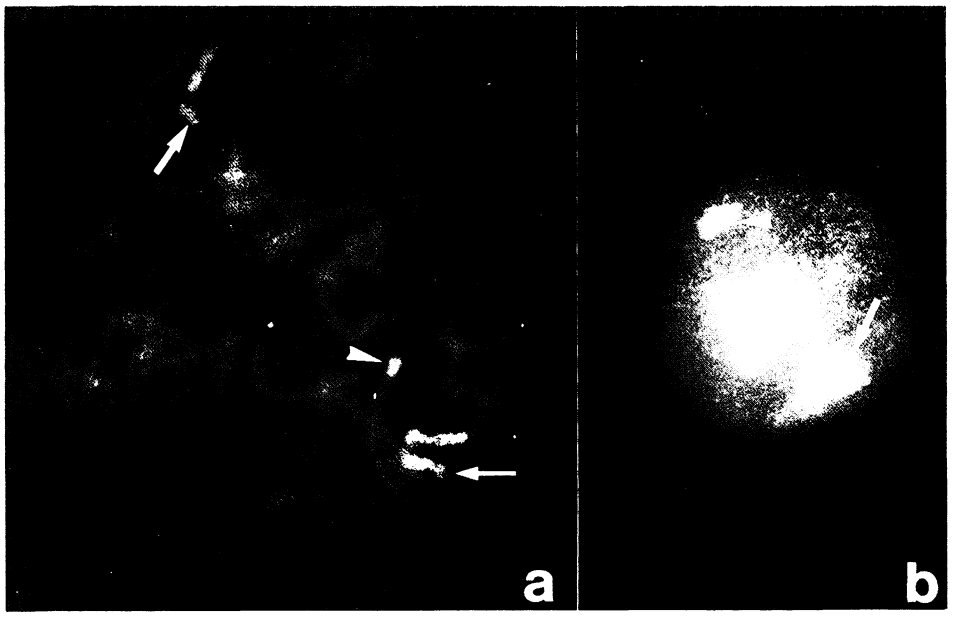

Fig. 1a, b : Prophase **(a)** and interphase nucleus **(b)** of a PHA-stimulated lymphocyte culture from a female individual with a reciprocal translocation t (1;9) (banding not shown) after CISS-hybridization with a biotinylated bacteriophage DNA library from sorted chromosomes 9 (LLO9NS01) ; kindly provided by the American Type Culture Collection (ATCC), detection with FITC-Avidin and counterstaining with propidium iodide. Microphotographs were taken with a Zeiss Photomicroscope III. In the prophase a normal chromosome 9 (thin arrow), a chromosome 9p- (thick arrow) and the translocated 9p- matrial (arrowhead) are clearly painted. The translocation is also obvious in the interphase nucleus, which shows a third small extra domain (arrowhead) in addition to the two chromosomes 9 and 9 p- domains (arrows).

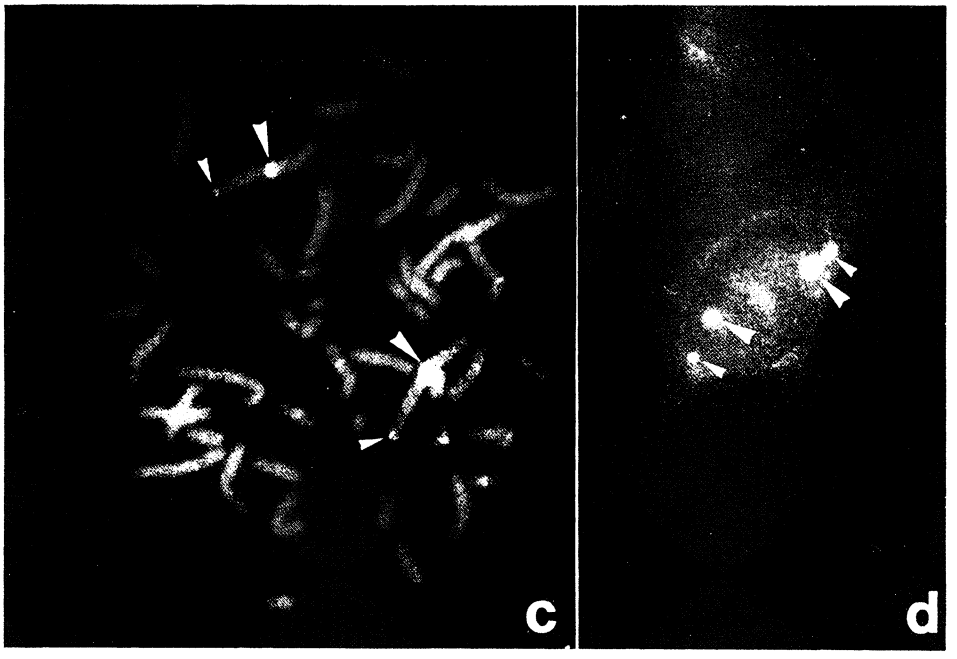

Fig. 1c, d : Lymphocyte metaphase spread (c) and interphase nucleus (d) from a male human fibroplast culture after fluorescence in situ hybridization of bands 1q12 (probe pUC1. 77 ; Cooke and Hindley 1979) and 1p36 (probe p1-79 , Buroker et al. 1987). In (c) both probes were labelled with biotin and detected with FITC-avidin. Chromosomes were counterstained with DAPI. In (D) pUC 1.77 was labelled with aminoacetylfluorene (AAF) and detected via rhodamine conjugated antibodies (large arrowhead : red fluorescence), while the biotinylated p1-9 probe was detected as described (small arrowhead : green fluorescence). Microphotographs were taken by double exposures with appropriate filters. For better preservation of morphology, the nucleus was fixed with 4 % paraformaldehyde and never air dried during the in situ hybridization procedure. For further methodological details see Cremer et al. (1988b) and Popp et al. (1990b).

Pinkel et al. 1988) for the visualization of specific chromosomes or chromosome subregions using composite DNA probes of various complexity. We will consider several typical examples and present a conceptual outlook for future developments.

Fig. 1a shows a lymphocyte prophase spread from a healthy woman with a translocation t(1;9) after painting of chromosome 9. This woman came to our attention after she had given birth to a child with a chromosome 9p- syndrome. The deletion in the short arm of one chromosome 9, as well as the translocation of this material to the long arm of chromosome 1 (banding data not shown) can be seen. A corresponding interphase nucleus (Fig. 1b) clearly exhibits three chromosome 9 domains, two larger ones and one distinctly smaller one, representing the translocated domain.

Fig. 1c,d show a lymphocyte metaphase spread (c) and an interphase nucleus from cultured human fibroblasts (d) after fluorescence in situ hybridization with two chromosome 1 specific, tandem-repetitive DNA probes (for details see legend to Fig. 1). Note that the orientation and spatial extension of the short arm of chromosome 1 can be easily recognized in this cell nucleus.

Fig. 2 examplifies the analysis of a single gene for the presence of specific intragenic deletions by CISS-hybridization. Lymphocyte metaphase spreads were prepared from the mother of a boy with Duchenne muscular dystrophy, a severe X chromosomal recessive disorder. Molecular analysis of the boy's dystrophin gene by multiplex PCR and Southern blot hybridization with the cosmid clone cPT1 revealed a deletion of a DNA segment around exon 45 as the cause of his desease (Ried et al. 1990). In case that the same deletion could be demonstrated in one of the two X chromosomes of his mother, she would be a proven carrier of the disease. For easy identification the two X- chromosomes were painted in red fluorescence (Fig. 2a). In addition, the intragenic region under consideration was visualized in green fluorescence with the cosmid clone cPT1 (Fig. 2b). Specific hybridization signals can clearly be seen on both chromatids at the expected region (Xp21) of the two X chromosomes. For further methodological details and discussion see Ried et al. (1990).

TWO DIMENSIONAL IMAGE ANALYSIS OF PAINTED CHROMOSOMES

While painted chromosomes or chromosomal subregions can be recognized easily by eye, automated digital image analysis is important to reduce the human workload when thousands of cells per case and/or a large number of cases have to be evaluated as for example in clinical applications of interphase cytogenetics (Cremer et al. 1986, 1988a, b ; Devilee et al. 1988 ; Hopman et al. 1989 ; Kolluri et al. 1990) or in case of biological dosimetry for persons with known risks of radiation exposure (Lucas et al.1989 ; Popp et al. 1990a). The desired features have to be extracted with high reliability by criteria such as number, morphology, color, etc., using methods of data averaging, background subtraction, filtering and segmentation. Cost effectiveness is another major point, if broad applications are envisaged. For such purposes we have started to develop a program called METSTAT written in the language C. It allows automatic contrast enhancement, segmentation and counting of painted chromosome regions. From the number of recognized signals and their size, metaphase spreads or interphase nuclei can be automatically classified as "normal" or "aberrant" ; for details see Popp et al. (1990a). In the present experiments the time needed for classification of a digitized image was 1-2 seconds using an IBM compatible personal computer with an INTEL 80386 microprocessor.

As an example Fig. 3a shows a lymphocyte metaphase spread from a healthy

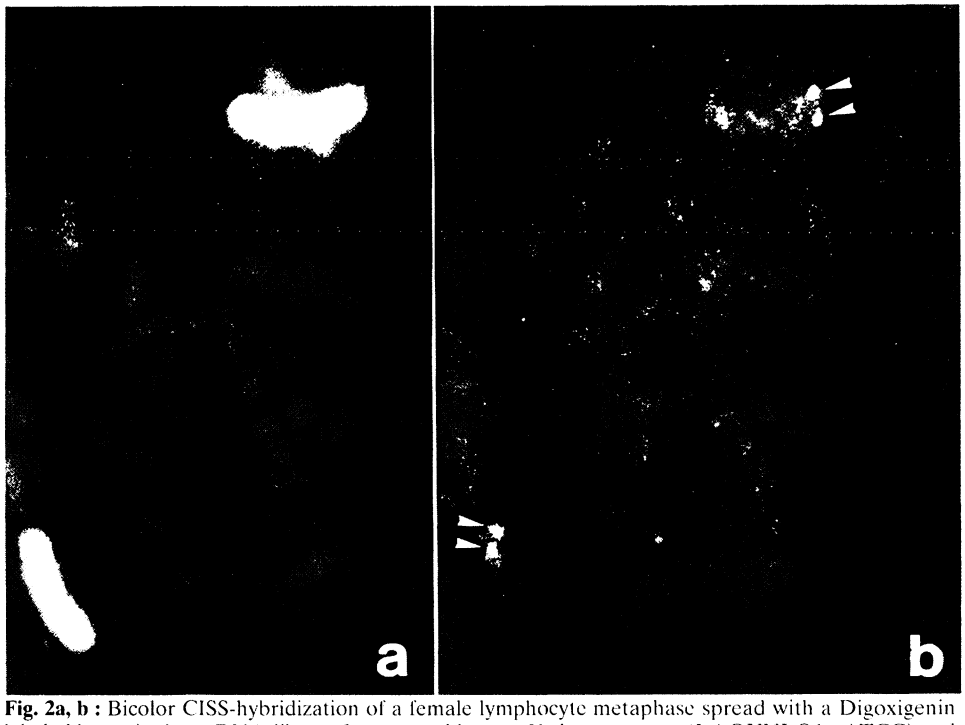

Fig. 2a, b : Bicolor CISS-hybridization of a female lymphocyte metaphase spread with a Digoxigenin labeled bacteriophage DNA-library from sorted human X chromosomes (LAOXNLO1 : ATCC) and cosmid clone cPT1 (see text : kindly provided by GJB can Ommen). The two X-chromosomes are painted in red fluorescence (a), while the region of the dystrophin gene detected by cPT1 is visualized by green dots on both chromatids of each of the two X-chromosomes (b : arrowheads). (Courtesy of M. Cremer and V. Mahler).

Fig. 2c : Digitized image of the metaphase spread shown in (b) photographed from a computer monitor after application of a Laplacean Filter and quadratic contrast enhancement.

Fig. 2d : The same image after automatic segmentation of the cosmid cPT1 signals.

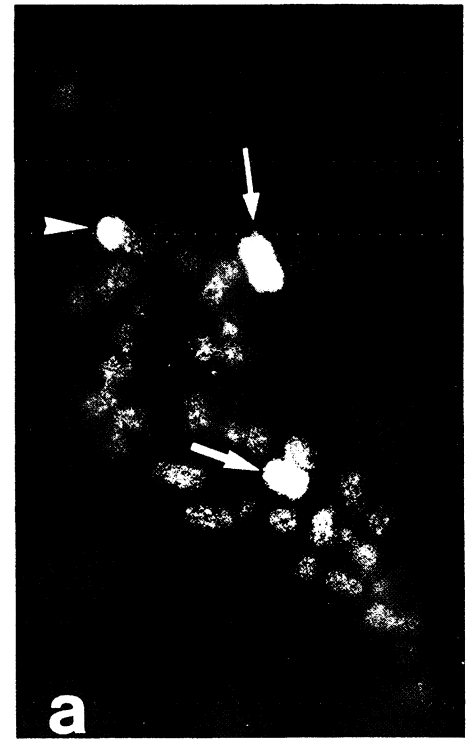

Fig. 3a : Lymphocyte metaphase spread demonstrating a spontaneous chromosome 1 translocation in a 63 years old healthy man after CISS-hybridization with a bacteriophage library from sorted human 1 chromosomes (LAO1NSO1 : ATCC) (from Popp et al. 1990 with permission). A normal chromosome 1 (thin arrow), a deleted chromosome 1 (thick arrow) and translocated chromosome 1 material (arrowhead) is indicated.

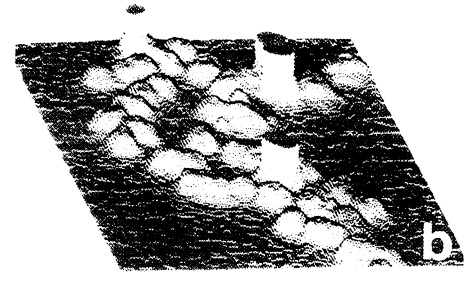

Fig. 3b : Pseudo 3D-image of the same metaphase spread after digitization, automatic contrast enhancement and segmentation of painted chromosomes.

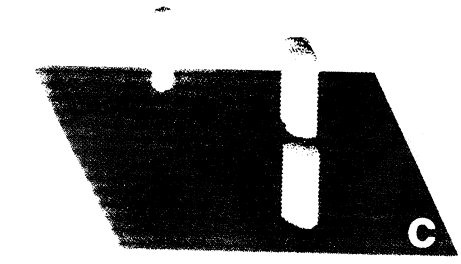

Fig. 3c : The same 3D-image after automatic background subtraction shows exclusively the number and localization of painted chromosomes.

male person with a spontaneous translocation of chromosome 1 material. After digitization (for details see Popp et al. 1990a) painted chromosome material could be separated from non-painted material due to their different gray levels without the need of additional filters (Fig. 3b, c). For the automated detection of hybridization signals from individual genes (Fig. 2c, d) a Laplacean filter matrix (Rosenfeld and Kak 1982) was applied to the digitized image using a 3 x 3 pixel mask with an empirically determined scaling factor S.

$$\begin{matrix} -1 & -1 & -1 \\ -1 & +S & -1 \\ -1 & -1 & -1 \end{matrix}$$

In the transformed images contrast was further enhanced using a quadratic look up table. After segmentation, all possible distances between the signals were automatically determined and normalized by METSAT. Two spots with a distance below an empirically determined threshold were considered as twin spots due to the hybridization of clone cPT1 to both chromatids of a given X chromosome.

In applications where large numbers of cells have to be analyzed, the velocity with which 2 D- images of interest can be identified and digitized for further analysis will likely become a serious bottle neck of automated analyses. Fast digitization of a microscopic specimen can be achieved via sensitive video — or CCD-camera systems (Lörch et al. 1989 ; Jovin and Arndt-Jovin 1989). If metaphase spreads are to be evaluated, a fast automated metaphase finder (Finnon et al. 1986 : Lörch et al. 1989) should be included in the system.

Problems for the automated detection of true hybridization signals from individual

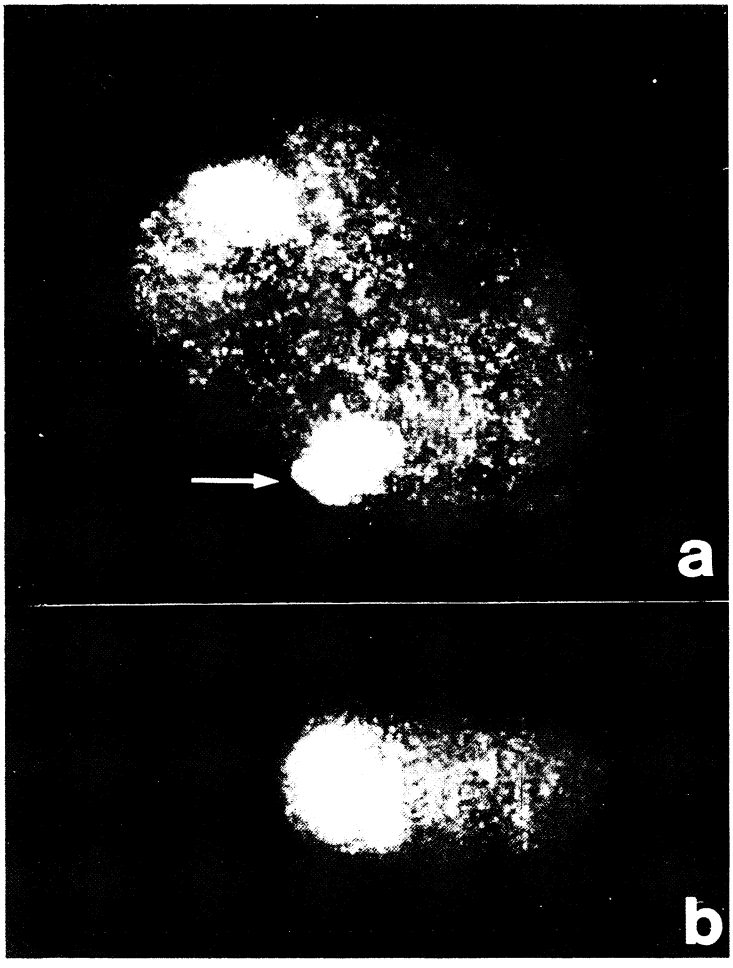

Fig. 4a, b : Horizontal (a) and vertical section (b) performed with a laser confocal scanning microscope through a female amniotic fluid cell nucleus after painting of the two X-chromosome domains with a biotinylated library from sorted human X chromosomes and counterstainting with propidium iodide. Arrow in (a) indicates the site where the vertical section was performed through the larger X-domain. Photographs were taken from the monitor. The height of the nucleus at the site shown in (b) is 7µm.

DNA clones will result, if the respective signals are small and have to be discriminated from scattered background dots. In such cases digital image analysis can be facilitated by simultaneous painting of the target chromosome in a different color (compare Fig. 2a, b). The latter, considerably stronger and more extended signals can easily and unequivocally be segmented. In this way the area of interest, which should be searched for the weak signal of an individual DNA clone, can be largely reduced.

THREE-DIMENSIONAL IMAGE ANALYSIS OF PAINTED CHROMOSOMES

While 2D-approaches as described above are appropriate for most diagnostic purposes, in situ hybridization in combination with three-dimensional digital microscopy has become a promising approach to answer basic questions of the nuclear organization such as the size and three-dimensional shape of individual normal and aberrant chromosomes and their suprachromosomal arrangements (e.g. Manuelidis and Borden 1988 ; Popp et al. 1990 b ; Geiger et al. 1991) as well as the distribution of specific genes and their transcripts (for review see Lichter et al. 1991). To perform this kind of analysis, optical sections of cell nuclei can be performed with "conventional" microscopy (Agard and Sedat 1983 ; Erhardt et al. 1985) or with a confocal laser scanning microscope (CLSM) (Cremer and Cremer 1978 ; Brakenhoff et al. 1979 ; for review see Shotton1988). Processing and visualization of complete optical sections, as well as the quantitative determination of important morphological features and the construction of 3D images are performed with the help of appropriate algorithms (Agard and Sedat 1983 ; Erhardt et al. 1985 ; Jones et al. 1990 ; Geiger et al., 1991).

Fig. 4 shows optical sections through a female amniotic fluid cell nucleus after painting of the two X chromosome domains with a DNA library from sorted human X chromosomes (for details of the treatment of nuclei for three-dimensional analysis and optical cell slicing see Popp et al. 1990 b and Seltzer et al. 1990). The horizontal section (Fig. 4a) demonstrates two X-domains, a vertical section through the larger one of these domains is shown in Fig 4b. Studies are presently underway in our laboratories to investigate possible differences in the three-dimensional shape and volume of the active versus the inactive X-chromosome in various human cell types.

OUTLOOK : MULTICOLOR IN SITU HYBRIDIZATION BANDING AND DIGITAL IMAGE ANALYSIS

Finally, the potential to develop completely new types of chromosome banding patterns on the basis of in situ hybridization techniques should be noted. In contrast to classical G-, R-, and C-Banding patterns the possibilities to create different in situ hybridization (ISH-) banding patterns seems practically unlimited. The number of chromosomal sites for which mapped DNA clones suitable for such experiments exist is rapidly increasing. In addition, microlibraries can be established from microdissected chromosomal subregions for painting of any desired subregion (Lengauer et al. 1991). Appropriate DNA clones may be combined in the future to create ISH-banding patterns according to the particular needs of each investigation, including whole chromosome sets, selected chromosomal subsets or individual mitotic or interphase chromosomes (for an example consider Fig. 1 c,d). Furthermore, the number, position, extension and relative signal intensity of individual ISH-bands in these patterns may be adapted to the particular needs of automated image analysis. The versatility and sensitivity of such an approach will be further improved by the implementation of multifluorescence color in situ hybridization protocols (Nederlof et al. 1990) which allow the simultaneous staining of multiple chromosome targets in different colors. In the course of these developments images have to be recorded with different optical filters. For further image analyses the resulting gray value arrays have to be aligned carefully by appropriate software using internal reference points. In addition, any automated analysis of banding patterns has to deal with the problem that the contraction of mitotic chromosomes varies largely for both biological and technical reasons. Recently, an algorithm (Fournier-Warping) has been developed to automatically extract the mean position, width and density of each band from a sample of chromosomes (Maurer and Wienberg 1991). In combination with these expected advances of ISH-banding this procedure may facilitate both the precise physical mapping of DNA clones along normal chromosomes and the analysis of structural chromosome changes.

REFERENCES

1 AGARD D.A. and SEDAT J.W. — Three-dimensional architecture of a polytene nucleus. Nature, 302 : 676-681 (1983)

2 AGARD D.A., HIRAOKA Y., SHAW P., SEDAT J.W. — Fluorescence microscopy in three dimensions. Meth. Cell Biol. 30 : 353-377 (1989)

3 ANASTASI J., LE BEAU M.M., VARDIMAN J.W., WESTBROOK C.A. — Detection of numerical chromosomal abnormalities in neoplastic hematopoietic cells by in situ hybridization with a chromosome-specific probe. Am. J. Pathol. 136 : 131-139 (1990)

4 BRAKENHOFF G.J., BLOM P. and BARENDS P. — Confocal scanning light microscopy with high aperture lenses. J. Microsc. 117 : 219-232 (1979)

5 BUROKER N., BESTWICK R., HAIGHT G., MAGENIS R.E., LITT M. — A hypervariable repeated sequence on human chromosome 1p36. Hum. Genet. 77 : 175-181 (1987)

6 CASPERSSON T., FARBER S., FOLEY G.E., KUDYNOWSKI J., MODEST E.J., SIMONSSON E., WAGH U., ZECH L. — Chemical differentiation along metaphase chromosomes. Exp. Cell Res. 49 : 219-222 (1968)

7 COOKE H.J., HINDLEY J. — Cloning of human satellite III DNA : Different components are non different chromosomes. Nucleic Acids Res. 6 : 3177-3197 (1979)

8 CREMER C., CREMER T. — Considerations on a laser-scanning-microscope with high resolution and depth of field. Microscopia Acta (Stuttgart) 81 : 31-34 (1978)

9 CREMER C., LANDEGENT J., BRUCKNER A., SCHOLL H.P., SCHARDIN M., HAGER H.D., DEVILEE P., PEARSON P. and VAN DER PLOEG M. — Detection of chromosome aberrations in the human interphase nucleus by visualization of specific target DNAs with radioactive and non-radioactive in situ hybridization techniques : diagnosis of trisomy 18 with probe L1. 84. Hum. Genet. 74 : 346-352 (1986)

10 CREMER T., LICHTER P., BORDEN J., WARD D.C. and MANUELIDIS L. — Detection of chromosome aberrations in metaphase and interphase tumor cells by in situ hybridization using chromosome specific library probes. Hum. Genet. 80 : 235-246 (1988a)

11 CREMER T., TESIN D., HOPMAN A.H.N. and MANUELIDIS L. — Rapid interphase and metaphase assessment of specific chromosomal changes in neuroectodermal tumor cells by in situ hybridization with chemically modified DNA probes. Exp. Cell Res. 176 : 199-220 (1988b)

12 CREMER T., POPP S., EMMERICH P., LICHTER P. and CREMER C. — Rapid metaphase and interphase detection of radiation-induced chromosome aberrations in human lymphocytes by chromosomal suppression in situ hybridization. Cytometry 11 : 110-118 (1990)

13 DEVILEE P., THIERRY R.F., KIEVITS T., KOLLURI R., HOPMAN A.H.N., WILLARD H.F., PEARSON P.L. and CORNELISSE C.J. — Detection of chromosome aneuploidy in interphase nuclei from human primary breast tumors using chromosome-specific repetitive DNA probes. Cancer Res. 48 : 5825-5830 (1988)

14 ERHARDT A., ZINSER G., KOMITOWSKI D. and BILLE J. — Reconstructing 3-D light-microscopic images by digital image processing. Applied Optics. 24 : 194-200 (1985)

15 FEARON R. and VOGELSTEIN B. — A genetic model for colorectal tumorigenesis. Cell : 61 : 759-767 (1990)

16 FINNON P., LLOYD D.C., EDWARDS A.A. — An assessment of the metaphase finding capability of the cytoscan 110. Mutat Res 164 : 101-108

17 GEIGER B., KOMITOWSKI D., JAUCH A., HAUSMANN M., CREMER C. — Optical sectioning and 3d-image reconstruction to determine the volume of specific chromosome regions in human interphase cell nuclei. Optics, in press (1991)

18 HOPMAN A.H.N., PODDIGHE P.J., SMEETS W.A.G.B., MOESKER O., BECK J.L.M., VOOIJS G.P. and RAMAEKERS F.C.S. — Detection of numerical chromosome aberrations in bladder cancer by in situ hybridization. Am. J. Path. 135 (6) : 1105-1117 (1989)

19 JAUCH A., DAUMER C., LICHTER P., MURKEN J., SCHROEDER-KURTH T. and CREMER T. — Chromosomal in situ suppression hybridization of human, gonosomes and autosomes and its use in clinical cytogenetics. Hum. Genet. 85 : 145-150 (1990)

20 JONES S.J., TAYLOR M.L., BAARSLAG M.W., KROL J.J., MOSTERD B., MANS A., BRAKENHOFF G.J. and NANNINGA N. — Image processing techniques for 3-D chromosome analysis. J. Microsc. 158(2) : 235-248 (1990)

21 JOVIN T.M. and ARNDT-JOVIN D.J. — Luminescence digital imaging microscopy. Annu. Rev. Biophys. Biophys. Chem. 18 : 271-308 (1989)

22 KOLLURI R.V., MANUELIDIS L., CREMER T., SAIT S., GEZER S. and RAZA A. — Detection of monosomy 7 in interphase cells of patients with myeloid disorders. Am. J. Hematol. 33(2) : 117-122 (1990)

23 LANDEGENT J.E., JANSEN IN DE WAL N., DIRKS R.W., BAAS F. and VAN DER PLOEG M. — Use f whole cosmid cloned genomic sequences for chromosomal localization by non-radiactive in situ hybridization. Hum. Genet. 77 : 366-370 (1987)

24 LENGAUER C., ECKELT A., WEITH A., ENDLICH N., PONELIES N., LICHTER P., GREULICH K.O., CREMER T. — Selective staining of defined chromosomal region by in situ suppression hybridization of libraries from laser-microdissected chromosomes. Cytogenet. Cell Genet., in press (1991)

25 LICHTER P., CREMER T., BORDEN J., MANUELIDIS L. and WARD D.C. — Delineation of individual human chromosomes in metaphase and interphase cells by in situ suppression hybridization using chromosome specific library probes. Hum. Genet. 80 : 224-234 (1988a)

26 LICHTER P., CREMER T., TANG C.C., WATKINS P.C., MANUELIDIS L. and WARD D.C. — Rapid detection of human chrosome 21 aberrations by in situ hybridization. Proc. Natl. Acad. Sci. USA. 85 : 9664-9668 (1988b)

27 LICHTER P., JAUCH A., CREMER T. and WARD D.C. — Detection of down syndrome by in situ hybridization with chromosome 21 specific DNA probes. In : Molecular Genetics of Chromosome 21 and Down Syndrome. New York, Alan R. Liss Publication (1990)

28 LICHTER P., BOYLE A.L., CREMER T., WARD D.C. — Analysis of genes and chromosomes by non-isotopic in situ hybridization. Genet. Anal. Techn. Appl. in press, 1991

29 LORCH T., WITTLER C., STEPHAN G., BILLE J. — An automated chromosome aberration scoring system. In : Automation of cytogenetics (C. Lundsteen, J. Piper (eds.)). Springer Verlag, Berlin, pp. 19-30 (1989)

30 LUCAS J.N., TENJIN T., STRAUME T., PINKEL D., MOORE D., LITT M. and GRAY J.W. — Rapid human chromosome aberration analysis using fluorescence in situ hybridization. Int. J. Radiat. Biol. 56(1) : 35-44 (1989)

31 LUNDSTEEN C., PIPER J. (eds.) — Automation of cytogenetics. Springer verlag, Berlin (1989)

32 MANUELIDIS L. and BORDEN J. — Reprodutible compartimentalization of individual chromosome domains in human CNS cells revealed by in situ hybridization and three-dimensional reconstruction. Chromosoma. 96 : 397-410 (1988)

33 MAURER A., WIENBERG J., FOURIER-WARPING — A new method to extract the mean features from chromosome banding patterns. Cytometry, in press (1991)

34 NEDERLOF P.M., VAN DER FLIER S., WIEGANT J., RAAP A.K., TANKE H.J., PLOEM J.S. and VAN DER PLOEG M. — Multiple fluorescence in situ hybridization. Cytometry. 11(1) : 126-131 (1990)

35 PINKEL D., LANDEGENT J., COLLINS C., FUSCOE J., SEGRAVES R., LUCAS J. and GRAY J.W. — Fluorescence in situ hybridization with human, chromosome-specific libraries : detection of trisomy 21 and translocations of chromosome 4. Proc. Natl. Acad. Sci. USA. 85 : 9138-9142 (1988)

36 POPP S., REMM B., HAUSMANN M., LUHRS H., VAN KAICK G., CREMER T. and CREMER C. — Towards a cumulative biological dosimeter based on chromosome painting and digital image analysis. Kerntechnik. 55(4) : 204-210 (1990a)

37 POPP S., SCHOLL H.P., LOOS P., JAUCH A., STELZER E., CREMER C. and CREMER T. — Distribution of chromosome 18 and X centric heterochromatin in the interphase nucleus of cultured human cells. Exp. Cell Res. 189 : 1-12 (1990b)

38 RAAP A.K., DIRKS R.W., JIWA N.M., NEDERLOF P.M. and VAN DER PLOEG M. — In situ hybridization with Hapten-modified DNA Probes. In : Modern Pathology of AIDS and Other Retroviral Infections. Basel, S. Karger, pp. (1990)

39 RIED T., MAHLER V., VOGT P., BLONDEN L., VAN OMMEN G.J.B., CREMER T., CREMER M. — Carrier detection by in situ suppression hybridization with cosmid clones of the Duchenne / Becker muscular dystrophy (DMB/BMD)-locus. Hum. Genet. 85 : 581-586 (1990)

40 ROSENFELD A., KAK A.C. — Digital picture processing. Vol. 1 and 2, Acad. Press, New York (1982)

41 SHOTTON D.M. — Confocal scanning optical microscopy and its applications for biological specimens. J. Cell Sci. 94 : 175-206 (1989)

42 STELZER E.H.K., WIJNAENDTS-VAN-RESANDT R.W. — Optical cell slicing with a confocal fluorescence microscope : microtomoscopy. In : Confocal microscopy (T. Wilson, ed). Acad. Press, London, pp. 199-212 (1990)

43 WIENBERG J., JAUCH A., STANYON R. and CREMER T. — Molecular cytotaxonomy of primates by chromosomal in situ suppression hybridization. Genomics, in press (1990)