Automated detection of radiation-induced chromosome aberrations following fluorescence in situ hybridization

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Biological dosimetry/chromosome aberrations/ionizing radiation/fluorescence in situ hybridization

The cytogenetic detection of rare chromosome aberrations induced by ionizing radiation requires the evaluation of large numbers of cells. In this report, an image analysis program based on thresholding of grey level histograms is described for a rapid automated detection of chromosome translocations in metaphase spreads from human lymphocytes following irradiation and chromosome in situ suppression (CISS) fluorescence in situ hybridization (FISH). To classify a metaphase spread as "normal", "aberrant", or "excluded", a minimum medium time of less than two seconds was required on a general purpose Personal Computer. Using appropriately stained specimen, for the false classification rate an upper limit of about 10% was estimated. The upper limit for the rate of "excluded" cells was estimated to be about 11% (99% confidence ranges).

FISH-procedures allow to score chromosome aberrations also in the interphase nucleus. To apply simple threshold algorithms for segmentation of the FISH-stained nuclear areas, it is required that the grey level contrast is sufficiently high. The preliminary results presented here on the image analysis of human lymphocyte nuclei suggest the feasibility of such an approach.

INTRODUCTION

For the cytogenetic monitoring of ionizing radiation, it may be necessary to evaluate large numbers of cells.

E.g., Evans and coworkers1) investigated radiation-induced chromosome aberrations in nuclear-dockyard workers, analyzing a total of approx. 44000 lymphocyte metaphase spreads in about 200 persons. Furthermore, they estimated that an accurate detection of the cytogenetic effect of 10 rem (100 mSv) on the incidence of dicentrics in a single individual would necessitate examining of the order of 10000 cells. A consideration of the Poisson statistics indicates a sharp increase in the cells to be evaluated if still lower doses have to be determined. The reason for this is the low incidence of dicentrics2). To reduce the human workload, automated procedures have been developed for the scoring of dicentric chromosomes3-5). Although the monitoring of dicentrics is widely used for biological and practical reasons2), the study of other aberrations.
particularly translocations, may be indicated as well\textsuperscript{6,7}. The induction of dicentric chromosomes may likely result in genetically imbalanced cells during subsequent cell cycles. As a consequence, the affected cells may die. For this reason, biological dosimeters based on the scoring of dicentrics may be particularly useful to evaluate irradiation damage during the first cell cycles after an acute irradiation event. In contrast, irradiation induced reciprocal translocations normally do not result in gross genetic imbalances and the affected cells may therefore be expected to retain the same proliferative potential as normal cells. Accordingly, a biological dosimeter based on the scoring of reciprocal translocations should be particularly useful to monitor the effects of a single exposure even many years after such an event has taken place, as well as cumulative effects of multiple or chronic exposures. The statistical requirements (cells to be evaluated) for translocation scoring are estimated to be in a similar range as for dicentrics\textsuperscript{6}. In the first case, however, the time required for the visual banding analysis of metaphase spreads (identification of translocations) is increased about tenfold as compared to dicentric scoring. Attempts to apply automatic image analysis of banded chromosomes\textsuperscript{8,9,10} to translocation monitoring, so far have met with considerable problems. Therefore in routine applications, the study of radiation-induced translocations in banded metaphase spreads so far has played a minor role only. The development of a long-term (cumulative) biological dosimeter as outlined above has now become feasible by the advent of non-isotopic in situ hybridization [ISH].

Using appropriate DNA probes, which can be established e.g. from chromosome specific DNA libraries\textsuperscript{11,12,13}, selective staining of whole human metaphase chromosomes or of chromosomal subregions has been performed following ionizing radiation; dose-effect relationships have been obtained by microscopic observation\textsuperscript{6,7,14}. Here, a computer program will be described for the rapid automated evaluation of metaphase spreads following irradiation and chromosome in situ suppression (CISS-) hybridization\textsuperscript{15,16,17,18} of the whole DNA of a given chromosome type ("chromosome painting"). A minimum medium time of less than 2 seconds was required to classify a metaphase spread.

Chromosome aberrations can be scored not only in metaphase but also in interphase cells. Recently, it has been shown that ISH allows the detection of numerical changes, deletions, and rearrangements of chromosomes in the interphase nucleus\textsuperscript{19–28}. A first application of this new approach to the assessment of \textsuperscript{60}Co-\gamma-rays induced aberrations in peripheral human lymphocytes indicated its feasibility\textsuperscript{6}. For the doses investigated (0, 2, 4, 8 Gy), the interphase dose-response curves suggested a quadratic dependence on dose. In this report, we shall discuss some problems connected with the automated evaluation of interphase nuclei.

**MATERIALS AND METHODS**

**Data Set**

For image analysis, a data set of digitized grey level images of 148 metaphase spreads of human lymphocytes was used. The lymphocyte specimen had been prepared a) following \textsuperscript{60}Co-\gamma-irradiation\textsuperscript{6} and b) from a patient exposed to the \textsuperscript{232}Th containing X-ray contrast
medium Thorotrast\textsuperscript{7).} In the specimen, CISS-hybridizations had been performed with DNA-libraries from chromosomes \#1-5, 7; visualization of hybridized material was obtained by fluorescein isothiocyanate (FITC)-fluorescence. Counter staining of non-labelled chromosomes had been performed with propidium iodide (PI). Thus, hybridized chromosome regions were discriminated from non-hybridized ones due to their PI+FITC fluorescence. In the following, this will be addressed as CISS-FISH [chromosomal in situ suppression fluorescence in situ hybridization].

In addition, three digitized images were evaluated of interphase nuclei from human lymphocytes following irradiation with \textsuperscript{60}Co-\textit{γ}-rays (8 Gy) and CISS-FISH of \#1\textsuperscript{6).} To test the basic performance of the automated aberration detection protocol in the case of interphase nuclei 131 digitized images were analyzed from unirradiated human lymphocyte nuclei following fluorescence in situ hybridization [FISH] of a pericentromeric region of the X chromosome by hybridization with the DNA probe pXBR\textsuperscript{29).} Digitization was performed as described\textsuperscript{6,7)} from microphotographs on diapositive films using a drum scanning densitometer (Color Scandig 2605, Joyce Loeb); a maximum of 256 grey levels was distinguished.

\textbf{Digital image analysis}

In the digitized images, hybridized areas (FITC plus PI fluorescence) and non-hybridized areas (PI plus background FITC fluorescence) were distinguished due to their different grey levels without the need of additional filters. The digitized images were transferred to an IBM compatible personal computer with an INTEL 80386 microprocessor and a 25 MHz clock. For evaluation, a program was written in Turbo C2.O. This program was used in different versions (META 4, META 5, META 6, META 7).

\textit{Description of the basic evaluation program (META 4)}

Firstly, we shall describe the basic version META 4\textsuperscript{7):} META 4 can be divided into different parts, visualization, contrast evaluation, quadratic contrast enhancing, segmentation, counting and surveying, and classification.

1. \textit{Contrast evaluation}

Fig. 1 shows a typical grey-level histogram. On the ordinate, the frequency of image elements (pixels) is given as a function of the grey-level (0–255). Under the conditions used here, the hybridized areas are to be expected to have grey-levels below threshold value TH.

The contrast of painted and unpainted chromosomes in the digitized image of metaphase spreads may vary within a wide range in different experiments, depending both on the efficiency with which chromosome specific sequences hybridize to their respective target chromosome, and the efficiency with which nonspecific signals can be suppressed. A low contrast can severely disturb the segmentation routine, i.e., the setting of the threshold in a given metaphase spread which separates painted chromosome regions from non-painted ones. Therefore the algorithm analyzes the grey-level histogram and determines the positions of \(TH_1\) and \(TH_2\), where \(TH_1\) is the beginning of the grey-level histogram \(>0\), while \(TH_2\) is the first maximum of the grey-level histogram \(\leq 255\) (Example see Fig. 1).
The algorithm analyses the grey-level histogram (channels 0 to 255) and determines the position of TH₁ and TH₂, where TH₁ is the beginning of the grey-level histogram > 0, while TH₂ is the first maximum of the grey-level histogram < 255. The threshold TH for the segmentation of painted chromosome material was automatically determined by TH = TH₁ + (TH₂ − TH₁) * C₂, where C₂ is an empirically determined constant which depends on the system used for digitization of the image.

All image pixels with gray values below TH are considered to belong to painted chromosome regions.

2. **Quadratic contrast enhancement**

   The difference \( TH₁ − TH₂ \) is used to set a relation between a quadratic look-up table operation and the actual contrast. The grey value \( pic(x, y) \) of every pixel of the digitized image is then replaced by

   \[
   pic(x, y)_{\text{new}} = \frac{(pic(x, y)_{\text{original}})^2}{(TH₂ − TH₁ + C₁)},
   \]

   where \( C₁ \) is an empirically determined constant.

3. **Segmentation**

   For this new grey-level histogram the values \( TH₁, TH₂ \) are determined as described above.
The following rather simple algorithm was developed for the segmentation of painted chromosome material, non-painted chromosomes and image background and proved to be less time consuming than entropy methods\(^3\):

\[
TH = TH_1 + (TH_2 - TH_1)C_2
\]

where \(C_2\) is an empirically determined constant which depends on the system used for digitization of the image. All image pixels with grey values below \(TH\) are considered to belong to painted chromosome regions.

4. **Counting and surveying**

A "signal" is defined as a contiguous area of pixels with a grey value smaller than \(TH\). The image matrix is scanned column by column until the first pixel fulfilling this condition appears. Starting from this position, all directly neighbouring pixels having also grey values less than \(TH\) are evaluated. Summing up, these pixel positions allow to calculate the "center of gravity" for each signal. For further evaluation of the image, the grey values of the recognized areas are set to grey level 254 and the program starts again. If no more new areas are found, the counting routine lists up the number of signals, their area in number of pixels and their position.

5. **Classification**

From the number of recognized signals and their size, the metaphases are classified as "normal" or "aberrant". The program first determines the size of the largest signal of the image. All signals smaller than 1/10 of the largest area are considered to be artifacts due to the staining procedure or to segmentation errors and are consequently neglected. A metaphase spread classified as "normal" shows one or two signals, whereas an "aberrant" metaphase spread contains more than two signals. If the number of signals below the 1/10 threshold is above a certain limit (empirically chosen as 3 in the present series of images), the whole metaphase spread is discarded from the evaluation (classification: "excluded").

6. **Display**

In principle, the program may run without display on a monitor. If such a display is desired, two versions are available:

a) Representation of chromosome areas according to grey value (The lower the grey value the brighter the image), or with a colour code.

b) Pseudo 3D images. For a perspective view of the grey value distribution, a program was written with the following features: The horizontal x,y-axes correspond to the axes of the grey value image. The higher the value of the vertical z-axes, the lower is the grey value, i.e. the brighter is the image at the given x,y position. A value \(z(x,y)\) is printed only if \(z(x,y) \geq z(x,y-u): U=1, 2, \ldots, y-1\) (see Fig. 2 for determination of axes). The time for execution took about 1 sec. for display version (a) and 7 sec. for version (b).
Fig. 2. Pseudo 3-D images of metaphase spreads.

a) A normal metaphase spread of a Thorotrast patient (see [7]). The arrows point to the two painted chromosomes.

b) The same metaphase spread with painted areas visualized as "table mountains": All grey values below a certain threshold were set to zero.

c) Painted areas segmented by the automated threshold procedure.

d) An aberrant metaphase spread irradiated with 2 Gy of $^{60}$Co-$\gamma$ rays after CIE-hybridization. Large arrow: apparently intact chromosome; small arrow: deleted chromosome; arrowhead: translocation.

e) The same metaphase spread with painted areas visualized as "table mountains".

f) Painted areas segmented by the auto-threshold procedure.
**Program variations**

da) version META 5:

Following the quadratic contrast enhancement, the image is simplified in the following way: of any cluster of 4 pixels, the mean value is determined as the grey value for the new image matrix; segmentation of this new image is performed as in META 4.

b) version META 6:

For contrast evaluation, in addition to $TH_1$, $TH_2$, the image parameters: brightest value: (bright) and mean brightness (mean) are calculated. Then the new grey value is calculated according to

$$pic(x, y) = \left[ \frac{pic(x, y)_{\text{original}} \cdot C_3 + C_4 \cdot \text{bright}}{-C_3 \cdot \text{bright}^2 - C_5 \cdot \text{mean}/100} \right]^2 / (TH_2 - TH_1 + C_1)$$

where $C_3-C_6$ are empirically determined constants. The analysis of metaphase spreads presented here was performed with $C_2=10$, $C_3=610$, $C_4=36$, $C_5=70$.

c) version META 7:

In addition to the quadratic contrast enhancement of META 4, a Laplacian filter matrix\textsuperscript{31,32} was applied to the digitized image using a 3 X 3 pixel mask with an empirically determined scaling factor $S$:

$$\begin{bmatrix}
-1 & 1 & -1 \\
-1 & S & -1 \\
-1 & 1 & -1
\end{bmatrix}$$

Here, $S=8$ was chosen. The remaining procedure was the same as in META 4, except that metaphase spreads with more than 6 signals below the 1/10 area threshold were excluded.

The total program execution (without display but including reading of the image from disk) took a mean time of approximately two to four seconds per image according to the program version (see Results).

**RESULTS**

*Image analysis of metaphase spreads*

Fig. 2 shows two examples for the automated threshold segmentation of hybridized chromosome material in human lymphocyte metaphase spreads. In Fig. 2a a pseudo 3D-image is presented of a cell following CISS-FISH of chromosome 1 material. In the original microscope photograph, two brightly fluorescing chromosome 1 were observed indicating a cell with two obviously normal chromosomes chromosome 1. In this case the two chromosomes (arrows) are adjacent to each other and characterized by a low grey level, i.e. a high z-value. Fig. 2b shows the “painted” areas after manual segmentation. The z-value of the segmented areas was set to grey level zero; In the pseudo 3D image this resulted in two “table mountains”. The program then automatically determined the number (2) of the segmented areas and their size (Fig. 2c).
Thus, the cell was correctly classified as “normal” by the program.

Fig. 2d presents a pseudo 3D image of another metaphase spread following CISS-FISH of chromosome 1 material. Again, the “painted” areas are characterized by a low grey value (high z-value). In this case, however, only one normal chromosome 1 was found (large arrow). The other chromosome is obviously deleted (small arrow); the deleted material has been translocated to another chromosome (arrowhead). Fig. 2e shows the segmented areas as “table mountains”. The program META 4 automatically segmented the hybridized areas (Fig. 2f), determined the number and size of the segmented areas, and correctly classified the cell as “aberrant”. Table 1 shows the results of the automated classification of a set of 147 images of metaphase spreads following ionizing irradiation and CISS-FISH. The program versions META 4 to META 7 were applied to the same data set. In case of the version META 4 (prior to segmentation, quadratic contrast enhancement of grey level only), out of 147 images, seven were “excluded”. From the remaining 140 images, 138 (98.6%) were correctly classified either as “normal” or “aberrant”. “Correctly” means that the same classification was obtained by direct visual inspection of the original microphotographs by a cyto geneticist. Only 1 assignment was “false positive” i.e. the image was classified by META 4 as “aberrant” while the human observer did not; only 1 assignment was “false negative”, i.e. the image was classified by META 4 as “normal” while the human observer did not. Thus for META 4 a total false classification rate of only 1.4% was obtained for the data set analysed. The application of the program versions META 5 to META 7 to the same data set led to considerably higher “false positive” rates. For META 6 also the false negative rate was increased. The entire false classification rate increased from 1.4% (META 4) to 7.5% in case of META 5 (mean value formation after quadratic grey level enhancement), 15.7% in case of META 6 (linear quadratic contrast enhancement), and 29.3% in case of META 7 (additional Laplace filter).

Table 1

|       | normal | aberrant | excluded | false positive | false negative |
|-------|--------|----------|----------|----------------|----------------|
| META4 | 122    | 18       | 7        | 1 (0.7%)       | 1 (0.7%)       |
| META5 | 115    | 28       | 4        | 9 (6.1%)       | 2 (1.4%)       |
| META6 | 110    | 29       | 8        | 12 (8.2%)      | 11 (7.5%)      |
| META7 | 83     | 56       | 8        | 43 (29.3%)     | 0              |

Automated classification of 147 human lymphocyte metaphase spreads following $^{60}$Co-$\gamma$-irradiation and CISS-hybridization of chromosome. For technical reasons, the image of Fig. 2a was not included here.

Table 2 shows the medium program execution times obtained for the different program versions on the system used. Spot counting took up to 80% of the entire execution time and thus may be regarded as the time limiting step. The medium execution times were 1.9 sec. per image for META 4; 2.3 sec. for META 5; 2.9 sec. for META 6; and 4.2 sec. (due to Laplacean filtering) for version META 7.
Image analysis of interphase nuclei

For some examples, a preliminary image analysis was performed of human lymphocyte interphase nuclei. Fig. 3a shows the image of a nucleus from a non-irradiated lymphocyte culture after CISS-hybridization of two chromosome 1 domains. In 3b, the domains are represented using an appropriate offset and threshold. The entire nuclear area was segmented correctly (Fig. 3c) using a higher threshold.

In Fig. 3d the digitized image of a nucleus with an aberrant chromosome 1 pattern following ionizing irradiation is presented. In Fig. 3e, f, the hybridized areas and the entire nuclear area, respectively, are represented using appropriate thresholds.

In both images (3b, e) the major hybridization areas were segmented in an acceptable way. This suggests that threshold segmentation procedures may be used for a reliable classification of chromosome aberrations in interphase nuclei, if a bright contrast between hybridized and non-hybridized areas is realized.

To test whether in such a case the classification procedures developed for the automated evaluation of metaphase spreads may also be used for interphase nuclei, a data set of 131 images of human female lymphocyte nuclei was used. In these nuclei the centromeric region of the X chromosome was FISH-labelled with a repetitive probe (pXBR). In the majority of cases, this
resulted in two bright hybridization spots per nucleus (Fig. 4a). The application of program META 4 led to the identification of two spots (Fig. 4b) and the correct classification "normal". Table 3 shows the results of the automated evaluation of the nuclear images. For both program versions META 4 and META 5, the false negative classification rate was low (0%) and (1.5%), respectively. The false positive rate was 10/131 (7.6%) for META 4 and 17/131 (13%) for META 5. The "correct classification" rate (88+13−10)/(131−30): (90%) appeared again to be somewhat better for META 4 than for META 5 (105/124: (85%). However, the exclusion rate was higher for META 4 (30/131; 23%) than for META 5 (7/131; 5.3%).

Table 3

|        | normal | aberrant | excluded | false positive | false negative |
|--------|--------|----------|----------|----------------|----------------|
| META4  | 88     | 13       | 30       | 10             | 0              |
| META5  | 112    | 12       | 7        | 17             | 2              |

Automated classification of 131 female human lymphocyte interphase nuclei following hybridization of an X chromosome region with the repetitive DNA probe pXBR (normal: n=2 spots identified; aberrant: n=1 or n>2; false positive: classified by the program as aberrant; false negative: classified by the program as normal, contrary to visual inspection).

Fig. 4. Analysis of a female lymphocyte nucleus following FISH with a repetitive DNA probe (pXBR), specific for the centromeric region of the X-chromosome. a) Microphotograph of a nucleus before digitization. The two hybridized chromosome regions are visible as bright yellow spots. b) Segmentation of hybridized areas with an automatic threshold procedure (threshold=40). c) Segmentation of the entire nucleus with threshold=80.

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DISCUSSION

Image analysis of metaphase spreads

Staining of entire individual human chromosomes or parts thereof by using fluorescence in situ hybridization (FISH) has provided a powerful means for the rapid detection of numerical and structural aberrations. The image analysis results presented here indicate that using simple threshold algorithms, a rapid automatic classification of metaphase spreads with radiation-induced chromosome aberrations is feasible. The basic idea of the program development was the following:

After conventional staining procedures, the 2D-image analysis approaches used for the automatic detection of chromosome aberrations require the segmenting of all individual chromosomes of metaphase spreads. Following CISS-hybridization of a specific chromosome, however, a cell can be classified as "normal" or "aberrant" according to the number and size of decorated areas (see Fig. 2). Instead of 46 objects in a metaphase spread, only a few have to be segmented; the calculation of integrated density profiles and their evaluation (which may be very complex in case of banding) is eliminated. Thus, the automatic image analysis of metaphase spreads after CISS-hybridization is expected to be considerably faster (using the same hardware) than in case of conventional staining. It is obvious that a reliable performance of a rapid and consequently relatively simple classification algorithm may be expected only in case of appropriately stained specimen. Here, a data set of 147 metaphase spreads following CISS-hybridization was used which allowed a clear classification by eye. Four program versions (META 4-META 7) were tested. Each of these programs allowed an optimum segmentation of at least one individual image.

To test the reliability of the programs for an automated classification, they were applied to the same data set (Table 1). The best result was obtained with META 4: Only one cell out of 140 metaphase spreads (7 cells (5%) excluded) was classified "false positive" (FP), and one cell was classified "false negative" (FN). This leads to upper limits of a 99% confidence range of 5% for the FP-rate and the FN-rate, respectively. The upper limit for the rate of "excluded" cells was estimated to be about 11%. A constant FN rate can be compensated by using a larger sample of cells to reach statistical significance. Recently, it has been shown experimentally for the automated classification of radiation-induced dicentrics by slit scan flow cytometry, that even much higher FN-rates can lead to useful relative dose-effect curves.

The image analysis results of metaphase spreads suggest that in case of well stained specimen, a very simple procedure may lead to a better classification rate than more complex ones: The lowest false classification rate (1.4%) was achieved with program version META 4 (quadratic contrast enhancement) whereas the overall performance of programs with more sophisticated algorithms resulted in considerably higher false classification rates (up to about 30% for META 7 which includes Laplacean filtering). This hypothesis is compatible with the results of an automated classification procedure using a pyramid algorithm ("coarse to fine trading"): An analysis of 118 randomly selected images of the same data set as used here led to a false classification rate of 24% (using the same criteria) whereas the computation time (using a
80386 PC) was between 80 sec and 440 sec per image (T. Reuter, J. Dengler, C. Cremer, unpublished results). In contrast, the threshold algorithm META 4 used here was not only much more efficient but required also a medium execution time of less than 2 sec per image on a 80386 PC (Table 2).

Image analysis of interphase cells

Labelling of chromosomes by non-isotopic in situ hybridization has already been proven to be a useful adjunct in tumor cytogenetics of interphase nuclei. In addition, it has been shown that "interphase cytogenetics" may be also applied to the detection of radiation-induced chromosome aberrations in interphase nuclei. If large numbers of nuclei are to be evaluated, automated and rapid classification routines are highly desirable. The application of threshold algorithms requires that the stained areas to be segmented differ sufficiently in their grey values from the rest of the nucleus. As Fig. 3b, e indicate, this condition may be met.

As a basic test of the reliability of a rapid threshold oriented, automated classification of interphase nuclei the program versions META 4 and META 5 were applied to a data set of 131 nuclear images. These images had been obtained from female human lymphocyte nuclei following FISH of a centromeric region of the X-chromosome; nuclei were selected by eye for digitization according to clear FISH-signals. The results (Table 3) suggest that a rapid classification may be indeed feasible using thresholding routines. In the case of META 4, about 23% (30/131) of the nuclear images were automatically excluded. From the remaining 101 nuclear images, 90% (91/101) were classified "correctly", i.e. a human observer of the original images reached the same conclusion. The false positive (FP) rate was about 10% (10/101); of the 101 nuclei, no nucleus was classified "false negative" (FN: an aberrant pattern observed by eye was not detected by the system). From the numbers in Table 3, an upper limit of the 99% confidence range for the FN rate may be estimated to be about 5%. If the version META 5 was used, the exclusion rate dropped to about 5% (7/131); the FP rate, however, was increased to about 14% (17/124), whereas the FN rate remained low (2/124; upper limit for the 99% confidence range about 8%). Cells classified as aberrant by the program (i.e. the number of "true" aberrant cells plus the number of false positive cells) may be reinspected by another program or by eye for final classification. In case of META 4, this figure was about 18% (13 + 10/131) of the total numbers of nuclei in case of META 5, it was about 22% (12 + 17). This suggests that a considerable reduction of the human workload can be achieved by rapid threshold oriented classification routines not only of metaphase spreads but also in the case of appropriately stained interphase nuclei.

The detection of chromosome aberrations in interphase nuclei may have considerable advantages where it is difficult or laborious to obtain an appropriate number of metaphase spreads: Since cell culture is not required here, a major obstacle to routine applications of biological dosimetry to a large number of cases may be removed. Furthermore, other cell types (e.g. hair, skin, tissue) might also be included. This might allow to obtain a biological estimate for local exposure to radiation and other mutagenic agents.
Perspectives

The results obtained so far suggest that a rapid automated classification of chromosome aberrations in metaphase spreads and interphase cells may be feasible. For a practical implementation, however, in addition to optimal FISH-staining, various other conditions have to be met:

1. Fast finding of cells.

   In lymphocyte specimen, this appears to be no problem for interphase nuclei; in the case of metaphase spreads, hardware and software is needed for a fast finding and relocating. At the present state of the art, this requires several seconds per cell\(35\). Thus, the maximum rate is limited to \(\sim 10^3\) metaphase spreads per hour. If a very large number of cells has to be analysed, and if metaphase chromosomes are readily available, a rapid preenrichment of potentially aberrant chromosomes might be achieved by slit scan flow sorting\(36,37\) of chromosomes stained by in situ hybridization in suspension\(38\). To improve the sorting, fast algorithms for the on-line-digital evaluation of slit scan profiles are being developed\(40\)–\(42\).

2. In this preliminary study, the grey value images were obtained by digitization of color microphotographs. To accelerate the analysis to a rate comparable with the program execution time, digitization has to be made directly from the specimen. This may be achieved, for example, by a highly sensitive CCD-camera suitable for fluorescence imaging\(43\).

3. In the report presented here, attention was focused on the classification of images of individual nuclei. In case of an automated evaluation of a large number of nuclei, the automated identification not only of the hybridized areas but also of the nuclei themselves may be required; i.e. first the nuclear boundaries have to be determined before segmentation of the ISH-labelled areas within these boundaries can start. The examples given here (Fig. 3c, f) confirm that this can be realized with appropriate thresholds (here established manually). We have previously shown that such a determination may be performed also by an automated threshold selection procedure\(44\). The time required was about 1 sec per nucleus. With the algorithms available, automated classification of nuclei may be estimated to be limited to \(\sim 10^3\) nuclei per hour. This rate, however, might be accelerated considerably, e.g. due to parallel processing. For example, if the nuclear images would be evaluated by a multiprocessor system\(39\), the number of nuclei classified per hour should be increased up to the \(so+der of n\)-times the individual time; e.g., each of \(n\) microprocessors would classify \(10^3\) nuclei per hour.

ACKNOWLEDGEMENT

This work was supported by the Deutsche Forschungsgemeinschaft. We gratefully acknowledge S. Popp, A. Jauch and T. Cremer for performing the FISH-experiments used to obtain the data set included in the present study. We thank the German Cancer Research Center (DKFZ), Heidelberg, for the possibility to use a Joyce Loebl Scanning drum densitometer, and Dr. M. Hausmann (Heidelberg) for discussions.
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