ACTA STEREOL 1990; 9/2: 157-162
PROC 7WQIA FREIBURG I. BR. 1990
ORIGINAL SCIENTIFIC PAPER

METAFER2: IMPROVED APPLICABILITY AND EXTENDED SENSITIVITY OF BIOLOGICAL DOSIMETRY BY MEANS OF QUANTITATIVE IMAGE ANALYSIS

Ingeborg Grell-Büchtmann, Wolfgang Hoffmann, Tina Dannheim, Anna Heimers, Astrid Nahrmann, Heike Schröder, Inge Schmitz-Feuerhake, Paul Tomalik
Department of Medical Physics, FB 1, University of Bremen, 2800 Bremen 33, Fed. Rep. of Germany

ABSTRACT

The sensitivity of dose estimation after exposure to ionizing radiation by means of analysis of specific chromosomal aberrations (biological dosimetry) depends statistically on the number of metaphase plates analysed. For low-dose ranges about 1000-2000 cells are necessary. Thus this high extent of time needed is the main limiting factor. By use of an automated computer supported metaphase screening and a documentation of the coordinates the time of analysis can be reduced.
METAFER2 (Fa. Metasystems, Ladenburg, FRG) consists of a light microscope with a video camera and a AT-computer including a VGA-card.
The video images are analysed using a special software. The coordinates of all registrated metaphases can then be relocated and the metaphases be analysed interactively. Further documentation and statistical analysis of the data is supported.
As METAFER 2 reduces the time needed by 50 %, the number of cases may be doubled or in terms of sensitivity, the detection limit can be lowered.
Advantages and limitations of the system are discussed using results of biological dosimetry of four individuals. Two of them were radiated occupationally, the other two live in a region with high natural radioactivity.

KEYWORDS: Biological dosimetry, chromosome aberrations, digital image analysis, ionizing radiation, METAFER2

THEORY AND METHODS OF BIOLOGICAL DOSIMETRY

Biological dosimetry is a method for retrospective evaluation of an accumalated equivalent whole-body dose of radiation exposed humans. It is a method that was developed 30 years ago and which has been established as a standardized tool for dose estimation of people who were exposed to ionized radiation, for instance where the physical monitoring had been insufficent, or the degree of exposure was unknown, especially after an accident had happened.

It is the only method for the investigation of radiation events that happened several years before. Even more than 20 years later these radiation induced chromosome aberrations are found in survivors of the Hiroshima A-bomb (Randolph and Brewen, 1980).

The dose of the radiation can be estimated by an *in vitro* calibration curve by quantitative analysis of chromosome aberrations. Relevant chromosome aberrations are dicentric chromosomes and centric rings. Figure 1 shows schematically most of the chromosome and chromatide aberrations that may occur after radiation.

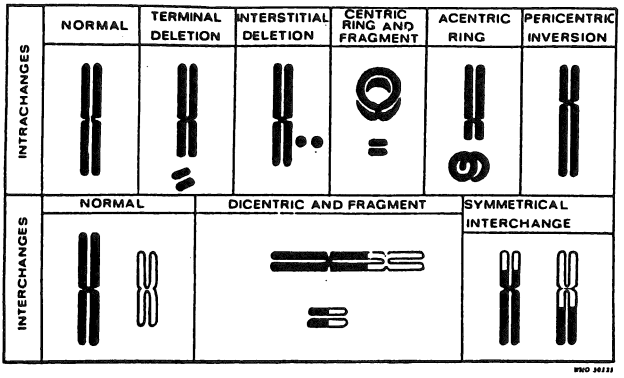

Fig.1: Chromosome and chromatide aberrations (from Buckton and Evans, 1973)

Dicentric chromosomes are formed from one double-strand break in two chromosomes close to each other where the broken ends fuse together. Centric rings are formed when two double-strand breaks in one chromosome fuse together. In both cases an acentric fragment remains.
Peripheral lymphocytes are preferred as a tool of investigation for chromosomal analysis for many reasons. They are easily obtainable, they circulate through the whole body, they do not divide anymore and they have a long lifetime.
Lymphocytes from peripheral venous blood are treated as described by Moorhead et al. (1960) and are dispensed on slides. Fluorescence-plus-Giemsa (FPG) staining is performed according to Perry and Wolff (1974) to obtain wellspread first division metaphases with clearly visible chromosomes.
Cytogenetic analysis is performed using a high-resolution light microscope.
Figures 2 and 3 show a typical normal cell type and a cell with radiation induced chromosome aberrations.

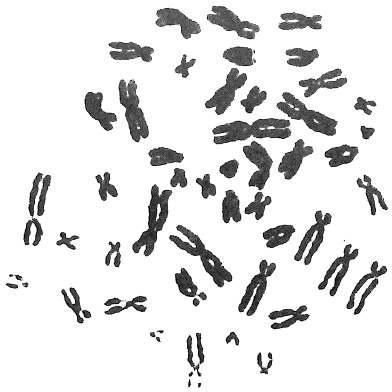

Fig.2: Metaphase plate with 46 chromosomes (normal cell type)

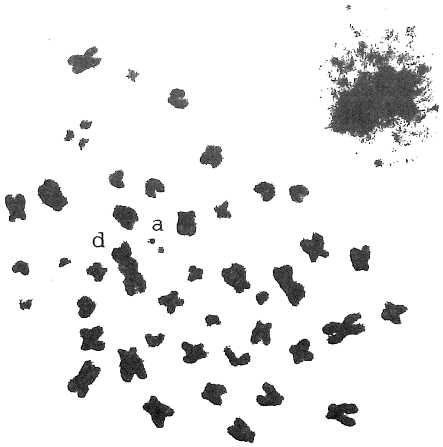

Fig.3: Metaphase plate with radiation induced chromosome aberrations (d=dicentric chromosome and a=acentric fragment)

Dicentric chromosomes, centric rings and acentric fragments can be analysed well in a light microscope. Their number corresponds directly to the dose of radiation.
Equivalent whole-body dose estimation is performed by means of an *in vitro* obtained calibration curve as the structural chromosome aberrations will be induced *in vitro* and *in vivo* in the same amount. Dose-response calibration curves has been established for different radiation qualities (Romm and Stephan, 1990).
Other influencing factors are dose rate and linear energy transfer (LET). For low LET the dose-effect curves correspond to a linear-quadratic relationship:

$$y = aD + bD^2 \qquad (1)$$

where y is the observed aberration rate (dicentric chromosomes/cell), D is the dose (Gray) and a (Gray exp.-1) and b (Gray exp.-2) are the regression coefficients.
The spontaneous rate of dicentric chromosomes in persons not exposed to radiation (back ground frequency) is very low, about 0.0004 dicentric chromosomes per cell (Bauchinger et al., 1983).
As there are some differences in culture conditions and individual scoring criteria each laboratory should establish its own calibration curves. Some other influencing factors might be mentioned in order to receive a reliable dose estimation:
1. The decline (time gap between exposure to radiation and analysis of the cells) of the aberrations is shown to follow an exponential function (Norman et al., 1966).
2. Partial-body exposure leads often to an underestimation as the degree of irradiated cells and unirradiated cells is not known. Also the kinetic of the lymphocytes is important.
3. It is important to know whether the exposure was chronical or acute, as the aberrations accumulate in the body.

DIGITAL IMAGE ANALYSIS

As the statistical relevance of the estimated equivalent whole-body dose on the 95% confidence limits depends on the number of scored cells, it is necessary to investigate a statistically sufficient number of acceptable metaphases (Kastenbaum and Bowman, 1970).
One of the most time consuming steps in the analysis of metaphases is the screening of the slides for metaphase positions. Every suitable position has to be measured exactly and the coordinates have to be documented to allow relocation and control.
This step could be fully automated by a computer-based quantitative image analysis named METAFER2 (Lörch and Stephan, 1986).
This system consists of a AT-computer with VGA-card, a light microscope supplied with a step-motorized stage and a video camera.
The microscope stage is directed by an extern hardware with several processors to allow precise movement (0.1 µm in x- and y-direction, 0.025 µm in focus direction).
The software consists of several sub-menues like metaphase screening, metaphase relocation, functions for coordinates, for the stage , for evaluation, and for application tuning.
To initialize the screening of the metaphase positions, maximal 8 slides can be placed on the stage. For any slide the user has to define the code, the area of search, several special classificators, and a typical reference metaphase to which the positions of all other metaphases are referred.The automatical screening begins first with the focusing of the slide at each point of intersection of the raster.
Second is the metaphase recognition in a hierarchic procedure of three steps, where the limits and areas may always be newly defined.
The hierarchic procedure is as follows:

1.step: any image field is analysed by use of a bandpass electronic for
- object count
- optical density
- filtered object count
- filtered optical density
- first gradient: sum of all 1-values of the first derivation of the video signal
- second gradient: sum of all 1-values of the second derivation of the video signal

2.step: from binary imagine the follwing parameters are calculated
- number of objects in a defined part of the image field
- average area of the objects
- average length of the objects
- average quotient of area to length
- number of objects per area of the metaphase

3.step: combination of the data leads to the decision of metaphase/no metaphase

Third is the calculation of the center of the metaphase in order to fix the coordinates.
After successful screening a protocol is printed for each slide including all parameters, a hardcopy of the reference metaphase and the number of metaphases. Depending on the amount of metaphases found the screening time varies from 20 to 40 minutes.

Now the user may relocate any metaphase and analyse the chromosomes interactively. For each metaphase it has to be decided whether it suffices the desired criteria. A special software menue allows a documentation of all aberrations and remarks of evaluated metaphases. Another special software allows the documentation of refused cells.
Additionally each coordinate can be transformed to another microscope using a calibration slide.
The data gained from the evaluation and the refusal represent the basis for statistical analysis.

EXAMPLES OF APPLICATION

As shown in table 1 our laboratory investigated four radiation exposed individuals. Two of them live in an area with high natural radon-222, that emanates from the subsoil. They are healthy siblings of children with leukemia (Hoffmann et al., 1990). The other two are occupationally exposed workers, one contaminated in a hospital the other in a nuclear power plant.

Table 1: Results of biological dosimetry of four individuals

cases	1	2	3	4
sex	male	female	female	male
kind of exposure	environ-mental	environ-mental	occupational medical therapy	occupational radiation accident
exposition	permanent	permanent	chronically	acute
period of exposure	12 years 1977-1989	15 years 1974-1989	7 years 1975-1982	2 hours 1984
screened cells	3817	3739	6259	2638
evaluated metaphases	1207 (32 %)	1095 (29 %)	1201 (19 %)	1203 (46 %)
dicentric chromosomes	-	1	3	3
centric rings	-	2	1	-
chromosome aberrations per metaphase	-	0.0027	0.0033	0.0025
estimated whole-body dose	-	nc	140-230 (mGy)*	110-190 (mGy)*
corrected for decline	-	nc	340-750 (mGy)#	340-750 (mGy)#

nc: not calculated
* : error of calibraton curve (Co-60) was taken into account
# : error of calibration curve and decline were taken into account

For statistical reasons at least 1000 metaphases were evaluated. As the quality of the metaphases differed strongly, the number of screened cells varied.
Dose estimation was performed only in cases 3 and 4 as the calibration curves are from Co-60 gamma radiation. The estimated whole-body dose has to be corrected for the decline. Also a partial-body radiation must be mentioned, so that the estimated doses are higher than those which could be analysed by biological dosimetry.
The usage of Metafer2 reduces the time of analysis by 50 %, so that the number of cells or the number of cases may be doubled or in terms of sensitivity and precision the increased number of analysed cells improves the detection limit.

ACKNOWLEDGEMENT

Parts of the work were supported by the Stefan-Morsch-Stiftung, Birkenfeld, FRG

REFERENCES

Bauchinger M, Schmid E, Streng S, Dresp J. Quantitative analysis of chromosome damage at first division of human lymphocytes after Co-60 Gamma irradiation. Radiat. Environ. Biophys. 22 (1983) 225-229.

Buckton KE, Evans HJ. Methods for the analysis of human chromosome aberrations. WHO Geneva 1973.

Hoffmann W. Kuni H, Artmann S, Bahr A, Götz A, Herrwerth C, Schmitz-Feuerhake I, Schubert F. Leukämiefälle in Birkenfeld und Umgebung: eine erste Bestandsaufnahme. Köhnlein W. (Hrsg.) Symposium Niedrigstrahlung und Gesundheit. Springer-Verlag Heidelberg 1990.

Kastenbaum M, Bowman KO. Tables for determing the statistical significance of mutation frequencies. Mutation Res. 9 (1970) 527-549.

Lörch T, Stephan G. Aufbau eines Systems zur automatischen Erkennung dizentrischer Chromosomen. ISH-Heft 89, Institut für Strahlenhygiene des BGA (1986).

Moorhead PS, Nowell PC, Mellman WJ, Battips DM, Hungerford DA. Chromosome preparations of leucocytes cultured from human peripheral blood. Exp. Cell Res. 20 (1960) 613-616.

Norman A, Sasaki MS, Ottoman RE, Fingerhut AG. Elimination of chromosome aberrations from human lymphocytes. Blood 27,5 (1966) 706-713.

Perry P, Wolff S. New Giemsa method for the differential staining of sister chromatids. Nature 251 (1974) 156-158.

Randolph ML, Brewen JG. Estimation of Whole-Body Doses by Means of Chromosome Aberrations Observed in Survivors of the Hiroshima A-Bomb. Radiation Research 82 (1980) 393-407.

Romm H, Stephan G. Chromosome analysis - a routine method for quantitative radiation dose assessment. Kerntechnik 55,4 (1990) 219-225.