Zoological Studies 34, Supplement I (1995)

Zoological Studies

# Three-dimensional Imaging Approaches and Monte Carlo Simulations: Development of Tools to Study the Morphology and Distribution of Chromosome Territories and Subchromosomal Targets in Human Cell Nuclei

Roland Eils[1,2], Kaan Saracoglu[2], Christian Münkel[1,3], Jorg Imhoff[1,3], Kurt Sätzler[1*,2], Etienne Bertin[4], Steffen Dietzel[5], Evelin Schröck[6], Thomas Ried[6], Thomas Cremer[5] and Christoph Cremer[2,1*]

[1]*Graduiertenkolleg "Modeling and Scientific Computing in Mathematics and Science" at 1*Interdisciplinary Center for Scientific Computing (IWR), University of Heidelberg, 69120 Heidelberg, Germany*

[2]*Institute of Applied Physics, University of Heidelberg, 69115 Heidelberg, Germany*

[3]*Institute of Theoretical Physics, University of Heidelberg, 69115 Heidelberg, Germany*

[4]*Equipe DyoGen and Lab. TIMC (CNRS URA 1618), Universite Joseph Fourier, Grenoble, France*

[5]*Institute of Human Genetics and Anthropology, University of Heidelberg, 69120 Heidelberg, Germany*

[6]*National Center for Human Genome Research, National Institute of Health, Bethesda, Maryland, USA*

Evidence has been accumulated indicating that the cell nucleus is compartmentalized both structurally and functionally. In particular, it has been demonstrated that chromosomes occupy distinct territories (Cremer et al. 1993). Chromosome territories and subchromosomal regions (e.g. genes) can be visualized by multiple color FISH and optical sectioning with a Leica confocal laser scanning fluorescence microscope. To study their structure in more detail we have developed truly three-dimensional imaging approaches. In addition, we describe Monte Carlo simulations which consider the distribution of such objects under the influence of various geometrical constraints. Such developments are essential in order to establish tools for refined studies of nuclear architecture.

## QUANTITATIVE IMAGE ANALYSIS USING THREE-DIMENSIONAL VORONOI DIAGRAMS

The image volume consisting of the whole stack of optical sections is first tessellated into polyhedra providing the Voronoi diagram (Bertin et al. 1993, Eils et al. 1995a). The neighborhood of the polyhedra is described by the dual Delaunay graph. Thereafter painted chromosomes consisting of connected polyhedra are extracted providing a three-dimensional segmentation of the image volume. The geometrical structure of the Voronoi diagram allows the rapid calculation of morphological parameters of segmented objects, such as volume, surface area, length and a shape factor. The construction of the Voronoi diagram for an image volume of a size of 256x256x32 is typically completed within 90 seconds on a Silicon Graphics Workstation (CPU R4000/100 MHz) (Eils et al. 1995a). The localization of subchromosomal targets within chromosome territories is described by two distance parameters. Firstly, the shortest distance d1 between the gravity center of the target and the surface of the tessellated chromosome territory is calculated. Secondly, the shortest distance d2 between the gravity center of the target and an inner line of the territory is territory. This inner line is obtained by the shortest path in the Delaunay graph connecting the two points with maximum distance in the chromosome teritory. The ratio D=d1/d2 provides a rough estimate for the more interior ($D \geq 1$) or more peripheral ($D < 1$) localization of the target within the chromosome territory. All parametrization steps described in this section are performed without any user interaction and typically completed for a given chromoscome territory in less than 10 seconds.

We have applied this technique to study the three-dimensional morphology of chromosome 7 and X territories. Volume ratios calculated for the two chromosome 7- and X-homologs in female human amniotic fluid cell nuclei were similar suggesting that the inactive X chromosome territory (Xi)

was not much more condensed than the active one (Xa). However, the Xi-territory had a ***significantly rounder shape and smaller surface than the Xa-territory (Eils et al. 1995b). These data are consistent with a recently proposed model predicting that the overall genetic activity of a chromosome territory is related to its surface (Cremer et al. 1993, Zirbel et al. 1993). To study the localization of genes within a given chromosome territory the c-myc protooncogene on chromosome 8 was visualized with its respective territory (Fig. 1). Figure 2 provides distance parameters as described above for the localization of this protooncogene in 14 chromosome 8 territories.

## ARTIFICIAL NEURAL NETWORKS FOR A CLASSIFICATION OF CHROMOSOME TERRITORIES

Artificial neural networks have been applied in an attempt to distinguish the inactive X-chromosome territory from the active one. 3D microscopic images of the chromosome territories serve as input for these networks. The artificial network consists of a layer of M input neurons and one output neuron. Each input neuron $i \in \{1...M\}$ processes the input data $\underline{b} = (b_1, b_2, ..., b_N) \in \Re_n$ and produces one bit $B_i$ of information (which is then passed to the output neuron):

$$B_i = \Theta\left(\sum_{j=1}^{N} J_{ij} b_j - \vartheta_i\right) \quad i \in \{1 \dots M\}$$

where $\Theta(x)$ is 0 for $x < 0$ and 1 for $x \geq 0$. The synaptic couplings $J_{ij}$ and the thresholds $\vartheta_i$ determine the output functions of the neurons. Equivalently, the one output neuron of the network collects the information of the input layer neurons and produces one output bit of the whole network:

$$B_{out} = \Theta\left(\sum_{j=1}^{N} \tilde{J}_i B_i - \tilde{\vartheta}_i\right)$$

In our case the input string b is a sequentially stored raw image of a X-chromosome territory. The network should decide whether this chromosome is active or inactive ($B_{out} = 0$ or 1, respectively). The network must be trained, i.e. the synaptic couplings and thresholds of the network must be adjusted to fulfill this task as good as possible. Well defined examples of active or inactive X-chromosome-territories (independently defined by Barr body staining) are used in the training algorithm. We have developed a novel training algorithm, called RECOMI (Repeated Correlation Matrix Inversion). RECOMI uses techniques of mathematical optimization theory and is able to optimize the stability of each neuron against noisy input, e.g. background fluorescence. Another major advantage of this algorithm is its high performance speed, since RECOMI does not use the images themselves, but only the image to image correlations. In 80%up to 90% depending on the amownt and set of training examples the network prforms a true

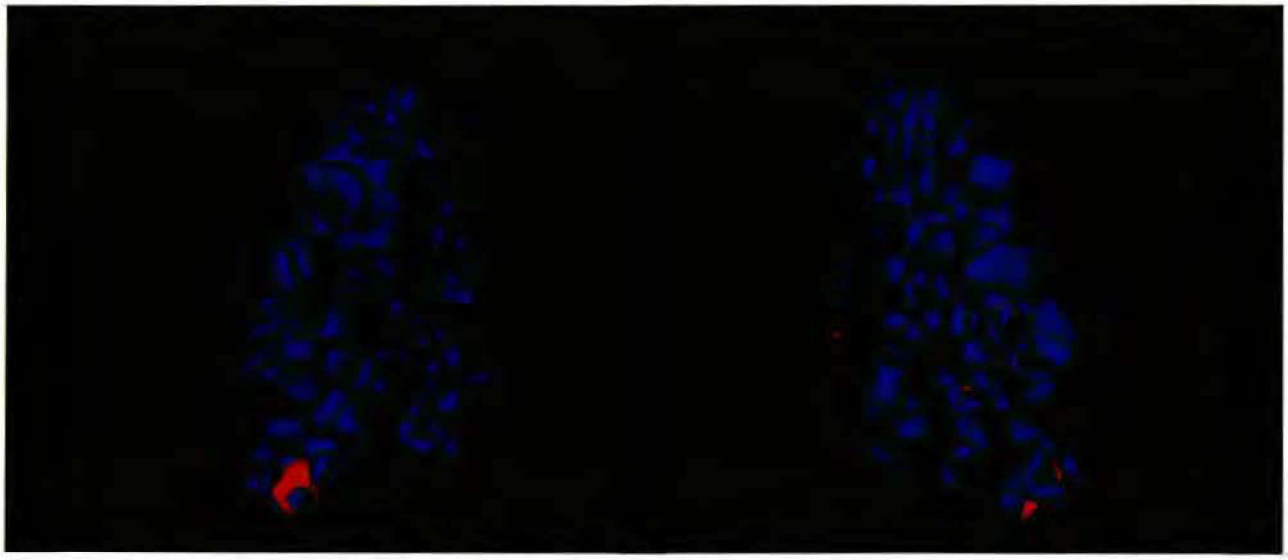

**Fig. 1.** Computer graphic visualization of a chromosome 8 territory (blue) painted with a chromosome 8 composite probe together with the chromatin domain harboring the c-myc protooncogene (red) hybridized with a phage-contig. The experiment was performed with a cultured, human female ammiotic fluid cell nucleus. The chromosome is shown from two sides (rotation 180 °)

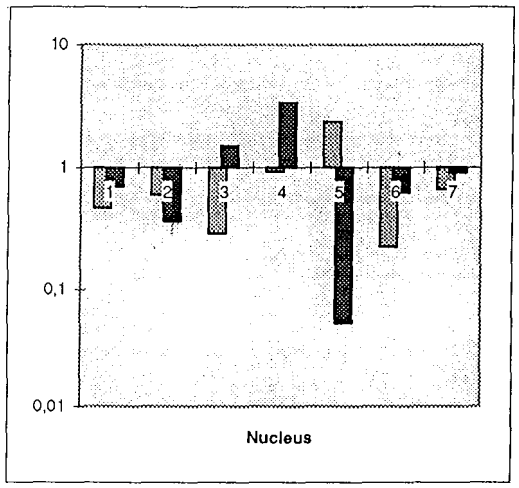


**Fig. 2.** Position of c-myc in 14 chromosome 8 territories determined by its D = d1/d2 ratio (for further explanation see text) in 7 diploid amniotic fluid cell nuclei (compare Fig. 1). Abscissa: Number of nucleus. Ordinate: Logarithm of the ratio values D. Note, that ratio values D smaller (larger) than 1 indicate a more peripheral (interior) localization of the target within the chromoscome territory.

classification of active or inactive x-chromosomes.

## MONTE CARLO SIMULATION OF THE DISTRIBUTION OF CHROMOSOMAL TARGETS IN CELL NUCLEI

The distribution even of "point like" subchromosomal targets in cell nuclei is affected by the spacious extension of chromosome territories. To study such geometrical constraints in a model system chromosome territories and cell nuclei were represented by ellipsoids and spheres. For comparison with experimentally observed distributions of point like targets, various assumptions were made with regard to their location either within or at the surface of model territories. For each assumption the distance between two targets, representing homologous chromosome regions, and between each target and the center of the model nucleus was calculated by Simple Sampling Monte Carlo simulations. Distributions of targets under the various constraints show strong deviations from a model, which assumes that pointlike targets are distributed uniformly and independently from each other within the nuclear space. Experimentally observed distributions of the pericentromeric heterochromatin of chromosome 17 in human lymphocyte nuclei by and large are compatible with the assumption that the respective three-dimensional distributions are solely affected by geometrical constraints. Distributions obtained for chromosome 7 heterochromatin, however, suggest that other than geometrical factors may be involved (Munkel et al. 1995).

To overcome possible drawbacks imposed by the shape rigidity of the geometrical model described above another model was developed which allows flexibility in chromosome territory shape. In this model each territory is composed by some hundred spherical subdomains. In a first step these subdomains are randomly located in a spherical model territory by hybrid Monte Carlo simulation. The volume of these model territories is directly related to the relative DNA-content of the respective chromosome. In a second step the volume of the model chromosome territories is reduced to some 15% allowing all 46 model territories of a diploid human chromosome complement to be placed randomly in the model nucleus. In the third step the volume of the model chromosome territories is increased stepwise by hybrid Monte Carlo simulation till they regain their original size. The simulation is continued to allow relaxation. The last two steps force an originally spherical model chromosome territory to form a more flexible shape. Neighboring territories obtain complementary shapes. The localization of point like targets in the original and transformed territory can also be simulated.

A hybrid Monte Carlo simulation for a nucleus with 46 territories required one day on an up-to-date workstation. To optimize performance, simulation was parallelized (geometrical parallelization and dynamic load balancing). Using four PowerPC (PowerXplorer, PARIX) the calculation time was reduced to approx. seven hours. The mean distance between the gravity center of the transformed chromosome territories and the center of the model nucleus is shown in Fig. 3. The data suggest that larger chromosome territories in comparison with smaller ones should be preferentially distributed toward the nuclear interior. More advanced models which take into account the additional restriction that each territory should be attached to the nuclear envelope are presently under consideration.

## REFERENCES

Bertin, E, F Parazza, JM Chassery. 1993. Segmentation and measurement based on 3D voronoi diagram: Application to

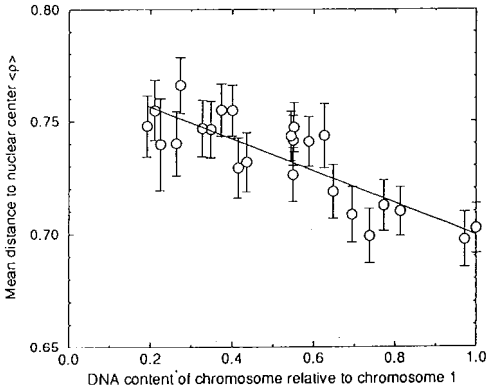


**Fig. 3.** Ordinate: Mean distances between the gravity center of chromosome territories (1-22, X and Y) and the nuclear center obtained by Monte Carlo simulation for 92 human model nuclei. Abscissa: relative DNA-content of each territory as compared to chromosome 1. Bars indicate mean distances obtained in a series of simulations. The line represents the linear regression curve.

confocal microscopy. Comput. Med. Imaging.Graph **17:** 175-182.

Cremer T, A Kurz, R Zirbel, S Dietzel, B Rinke, E Schröck, MR Speicher, U Mathieu, A Jauch, P Emmerich, H Scherthan, T Ried, C Cremer, P Lichter. 1993. The role of chromosome territories in the functional compartmentalisation of the cell nucleus. Cold spring Harb. Symp. Quant. Biol. **58:** 777-792.

Eils R, E Bertin, K Saracoglu, B Rinke, E Schröck, F Parazza, Y Usson, M Robert-Nicoud, EHK Stelzer, JM Chassery, T Cremer, C Cremer. 1995a. Application of laser confocal microscopy and 3D-Voronoi diagrams for volume and surface estimates of interphase chromosomes. J. Microsc. in press.

Eils R, S Dietzel, E Bertin, E Schröck, MR Speicher, T Ried, M Robert-Nicoud, C Cremer, T Cremer. 1995b. Active and inactive X-chromosome territories can be discriminated by surface and shape but not by volume. submitted.

Münkel C, R Eils, J Imhoff, S Dietzel, C Cremer, T Cremer. 1995. Simulation of the distribution of chromosome targets in cell nuclei under geometrical constraints. submitted.

Zirbel RM, UR Mathieu, A Kurz, T Cremer, P Lichter. 1993. Evidence for a nuclear compartment of transcription and splicing located at chromosome domain boundaries. Chromosome Res. **1:** 93-106.