Three - Dimensional Organization of the Human Interphase Nucleus
Experiments compared to Simulations

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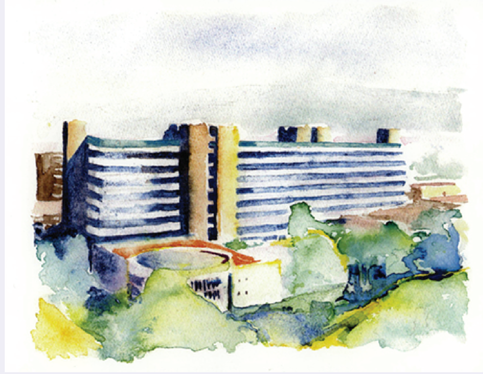
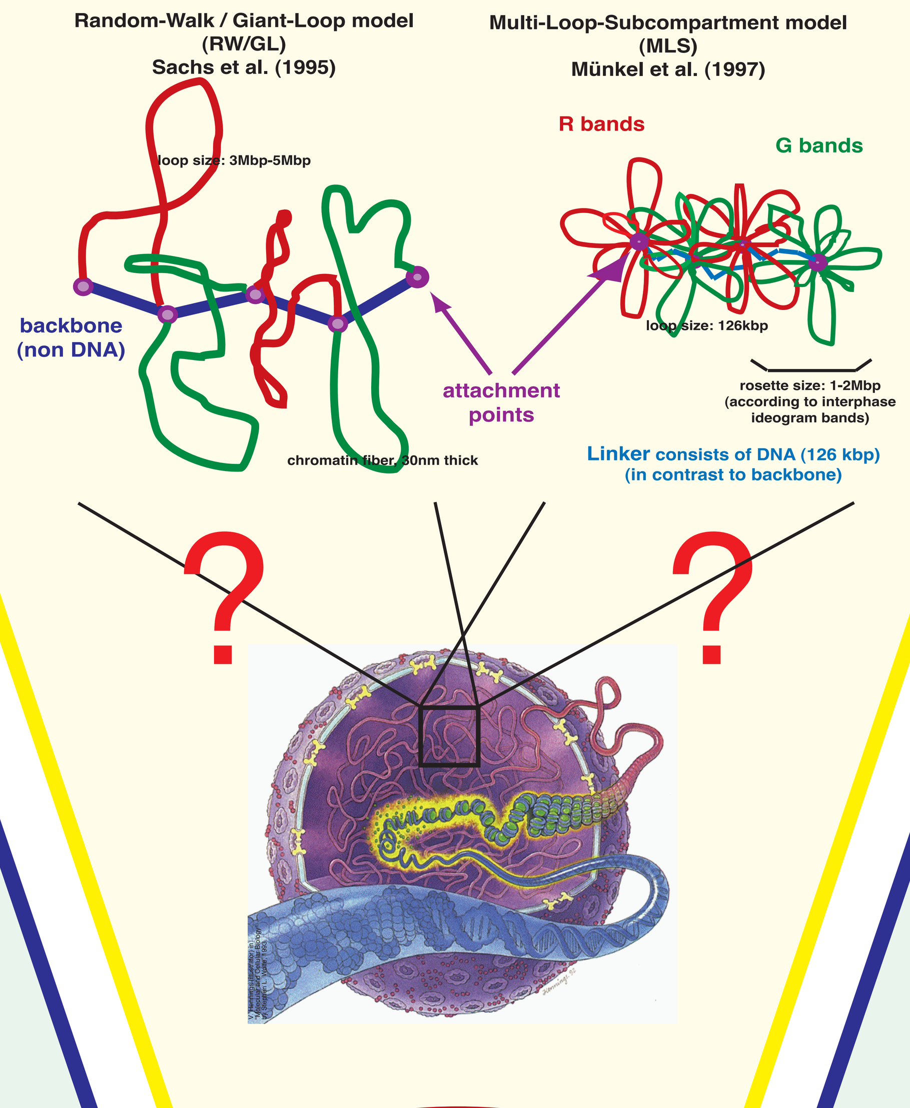


INTRODUCTION

Despite the successful linear sequencing of the genome its three dimensional structure is widely unknown although its importance for gene regulation and replication. Through a comparison between experiments and simulations we show here an interdisciplinary approach leading to the determination of the three- dimensional organization of the human genome.



FISH

Fluorescence in situ hybridization (FISH) is used for the specific marking of chromosome arms (Fig. 1A) and pairs of small chromosomal DNA regions (Fig. 1B). The labeling is visualized with confocal laser scanning microscopy followed by image reconstruction. Chromosome arms show only small overlap and globular substructures, as predicted by the MLS-model (Fig. 1A & 6A). A comparison between simulated and measured spatial distances between genomic regions as function of their genomic distances results in a good agreement with the MLS-model having loop sizes of arround 126 kbp and linker sizes between 63 kbp and 126 kbp (Fig. 2).

Fig. 1A & 1B: FISH-images of a territory painting of chromosome 15 (left, 1A) and genomic markers YAC-48 and YAC60 (right 1B) with a genomic separation of 1.0 Mbp in interphase of fibroblast cells.

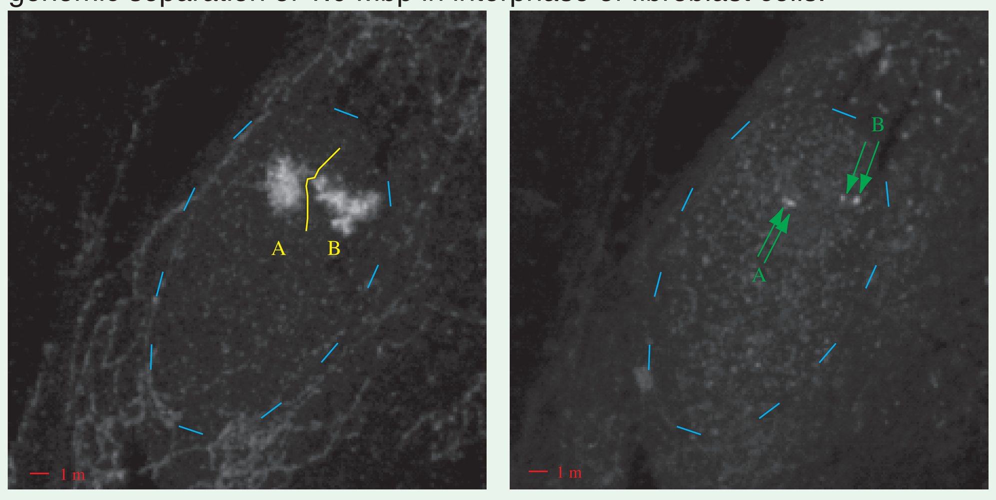
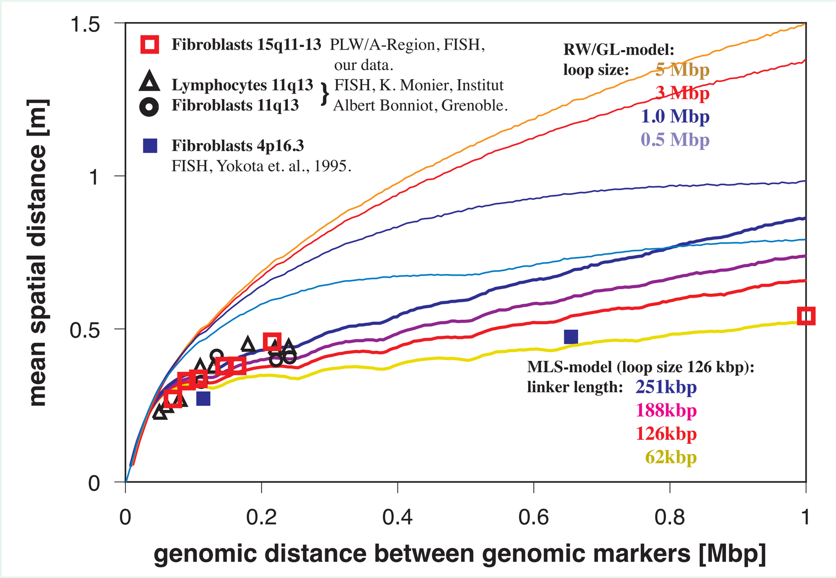


Fig. 2: Comparison of the RW/GL- and the MLS-model with experimentally determined interphase distances.



SIMULATION

For the prediction of experiments we simulated various models of human interphase chromosome 15 with Monte Carlo and Brownian Dynamics methods. The chromatin fiber was modelled as a flexible polymer fiber. Only stretching, bending and excluded volume interactions are considered. Chromosomes are further confined by a spherical potential representing the surrounding chromosomes or the nuclear membrane. Only the MLS model leads to clearly distinct functional and dynamic subcompartments in agreement with experiments (Fig. 6B & 1A) in contrast to the RW/GL models where big loops are intermingling freely and featureless (Fig. 6C & 6D).

Fig. 6A: Starting configuration with the form and size of a metaphase chromosome.

Fig. 6B: MLS model with 126 kbp loops and linkers. **Fig. 6C:** RW/GL model with 126 kbp loops. **Fig. 6D:** RW/GL model with 5 Mbp loops.

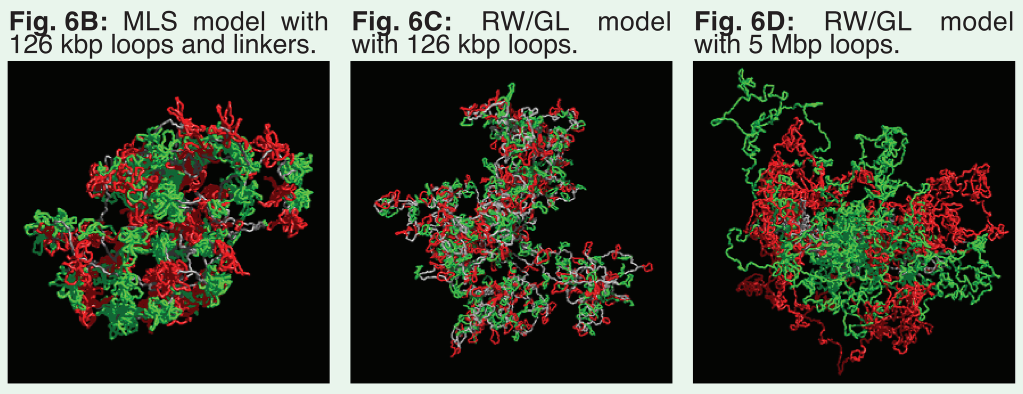
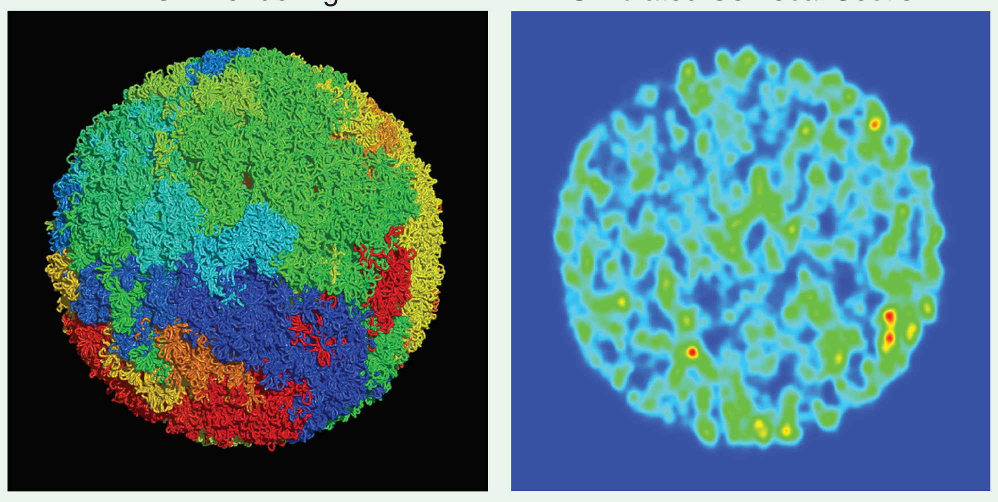


Fig. 7A & 7B: Simulation of a human interphase nucleus containing all 46 chromosomes with 1,200,000 polymer segments. The MLS-model leads to the formation of distinct and non-overlapping chromosome territories.

3D-Rendering — Simulated Confocal Section

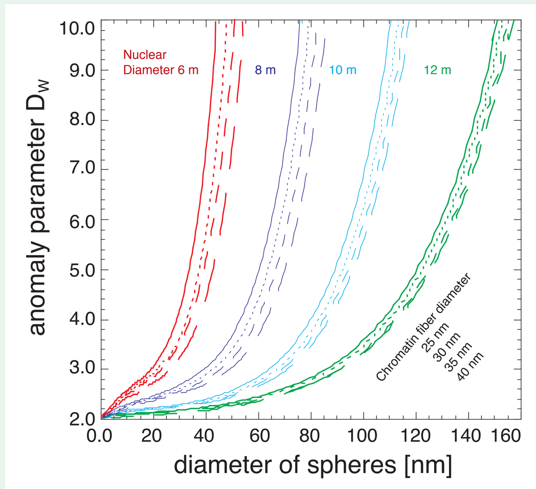


CONCLUSION

Simulations of chromsomes and the whole cell nucleus show that only the MLS-model leads to the formation of non-overlapping chromosome territories and distinct functional and dynamic sub-compartments. Spatial distances between FISH labeled pairs of genomic markers as function of their genomic distance agrees with an MLS-model with loop sizes of 120 kbp and linker sizes of 63 to 126 kbp. The in vivo chromatin distribution visualized by histone-GFP fusion proteins is similar to those found in the simulation of whole cell nuclei. Fractal analysis of the simulations reveal the multifractality of chromosomes. It is possible to quantify the in vivo chromatin distribution with fractal analysis and to relate the result to differences in morphology. The simulated diffusion of particles in the nucleus is only moderately obstructed by the chromatin fiber topology in agreement with FCS experiments. Simulated fragment distributions, based on double strand breakage after carbon-ion irradiation, differs in different models. Here again a comparison to experiments favours an MLS-model.

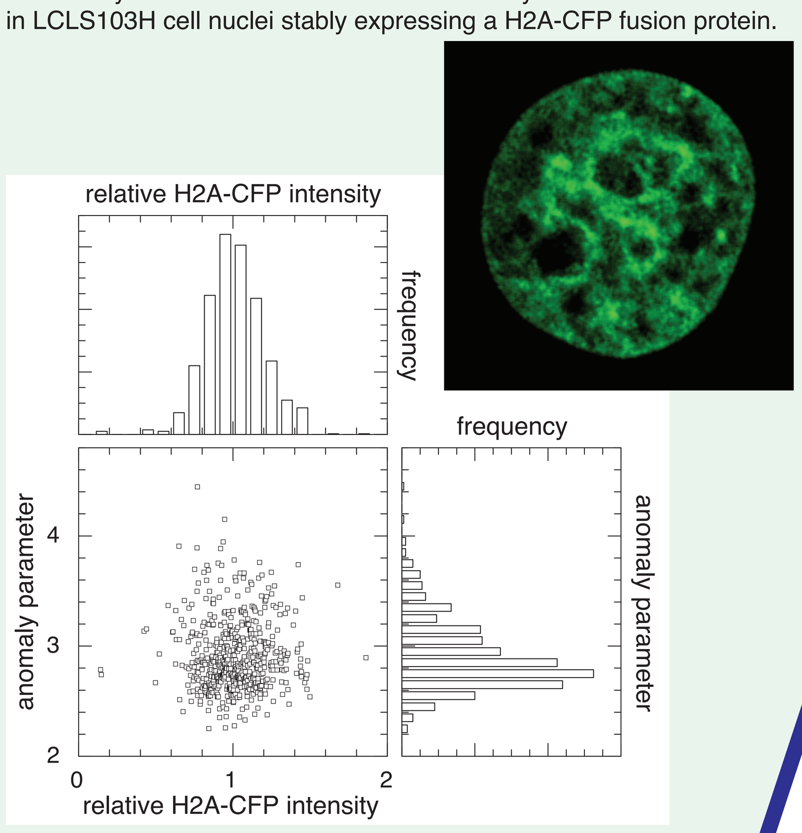
PARTICLE DIFFUSION

Fig. 3: Simulated obstruction of diffusion



The diffusion of spherical particles within a nucleus was simulated using Brownian Dynamics methods. The mean square displacement of the particles depends on the diameter, the radius of the nucleus, i.e. the obstacle concentration, and also critically on the interaction between particles and structure. For typical biological particles <10 nm the degree of obstruction D_W is moderate (Fig. 3). Thus such particles reach most nuclear locations in less than 10 - 20 ms. This agrees with the volume occupancy and mean chromatin fiber spacing. The diffusion of particles in living interphase nuclei depends on the local structure. In vivo chromatin markers allow to investigate this relation using fluorescence correlation spectroscopy (FCS). The correlation between diffusion obstruction and structure vanishes for small particles (Fig. 4) and increases with increasing particle size.

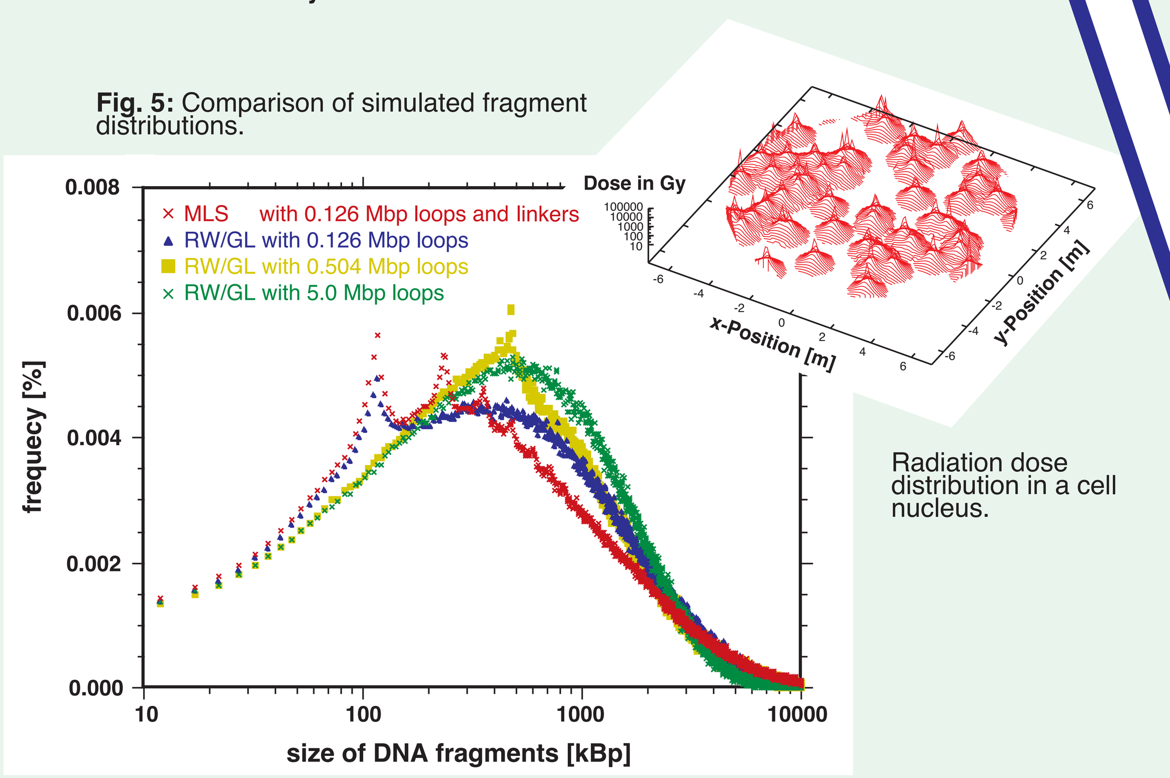
Fig. 4: The degree of diffusion obstruction plotted against the chromatin density, represented by the H2A-CFP fluorescence intensity. Data from FCS of Alexa568 dye in LCLS103H cell nuclei stably expressing a H2A-CFP fusion protein.



CARBON - ION IRRADIATION

The length distribution of DNA fragments after irradiation with carbon ions and the sites of double strand breakage depend on the spatial arrangement of the 30 nm chromatin fiber in the nucleus. Simulated configurations of different chromosome models were taken as the basis for the detailed simulation of these fragment distributions. The RW/GL-model and the MLS-model lead to clearly distinct fragment distributions (Fig. 5). A comparison with experiments favours an MLS-model. The specifity of breakage sites is currently analyzed. Data by P. Quicken.

Fig. 5: Comparison of simulated fragment distributions.



Radiation dose distribution in a cell nucleus.

FRACTAL ANALYSIS

Fractal analysis is especially suited to quantify the unordered and non-euclidean chromatin distribution of the nucleus. The dynamic behaviour of the chromatin structure and the diffusion of particles in the nucleus are also closely connected to the fractal dimension. The fractal analysis of the simulation of chromosome 15 lead to multifractal behaviour in agreement with porous network research (Fig. 8). Therefore chromosome territories show a higher degree of determinism than previously thought. First tests of fractal analysis of chromatin distributions by histone fusions to fluorescent proteins in vivo result in significant differences for different morphologies (Fig. 9) and might favour an MLS-model like chromatin distribution.

Fig. 8: Comparison of RW/GL- and MLS- model with fractal dimension of the chromatin fiber from simulations.

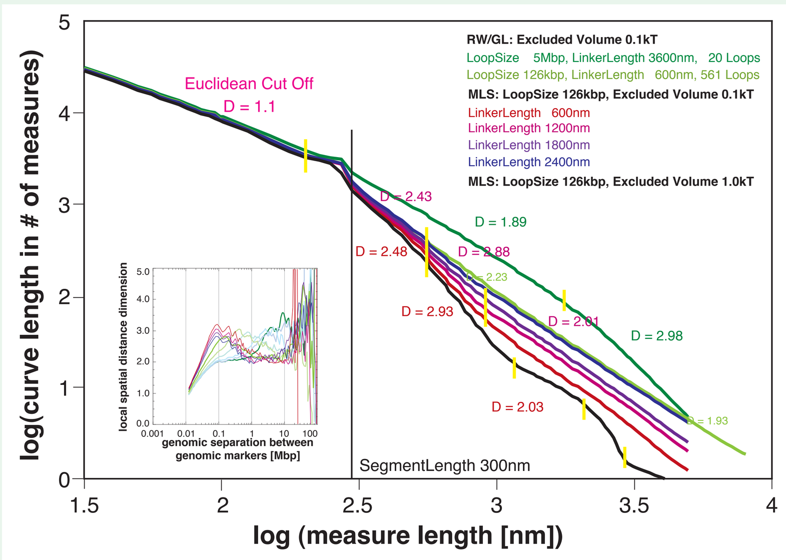
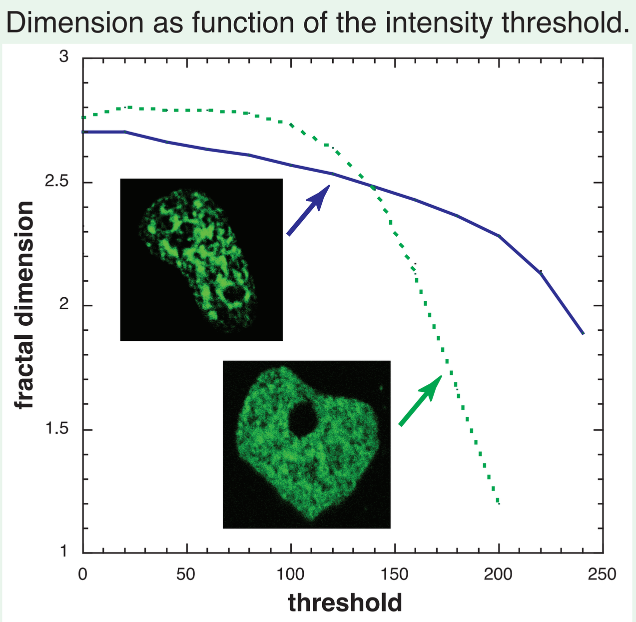


Fig. 9: Fractal Dimension as function of the intensity threshold.



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Abstract

To approach the three-dimensional organization of the human cell nucleus, the structural-, scaling- and dynamic properties of interphase chromosomes and cell nuclei were simulated with Monte Carlo and Brownian Dynamics methods. The 30 nm chromatin fibre was folded according to the Multi-Loop-Subcompartment (MLS) model, in which ~100 kbp loops form rosettes, connected by a linker, and the Random-Walk/Giant-Loop (RW/GL) topology, in which 1-5 Mbp loops are attached to a flexible backbone. Both the MLS and the RW/GL model form chromosome territories but only the MLS rosettes result in distinct subcompartments visible with light microscopy and low overlap of chromosomes, -arms and subcompartments. This morphology and the size of subcompartments agree with the morphology found by expression of histone auto-fluorescent protein fusions and fluorescence *in situ* hybridization (FISH) experiments. Even small changes of the model parameters induced significant rearrangements of the chromatin morphology. Thus, pathological diagnoses based on this morphology, are closely related to structural changes on the chromatin level. The position of interphase chromosomes depends on their metaphase location, and suggests a possible origin of current experimental findings. The chromatin density distribution of simulated confocal (CLSM) images agrees with the MLS model and with recent experiments. The scaling behaviour of the chromatin fiber topology and morphology of CLSM stacks revealed fine-structured multi-scaling behaviour in agreement with the model prediction. Review and comparison of experimental to simulated spatial distance measurements between genomic markers as function of their genomic separation also favour an MLS model with loop and linker sizes of 63 to 126 kbp. Visual inspection of the morphology reveals also big spaces allowing high accessibility to nearly every spatial location, due to the chromatin occupancy <30% and a mean mesh spacing of 29 to 82 nm for nuclei of 6 to 12 µm diameter. The simulation of diffusion agreed with this structural prediction, since the mean displacement for 10 nm sized particles of ~1 to 2 µm takes place within 10 ms. Therefore, the diffusion of biological relevant tracers is only moderately obstructed, with the degree of obstruction ranging from 2.0 to 4.0 again in experimental agreement.

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<u>*Keywords:*</u>

Genome, genomics, genome organization, genome architecture, structural sequencing, architectural sequencing, systems genomics, coevolution, holistic genetics, genome mechanics, genome function, genetics, gene regulation, replication, transcription, repair, homologous recombination, simultaneous co-transfection, cell division, mitosis, metaphase, interphase, cell nucleus, nuclear structure, nuclear organization, chromatin density distribution, nuclear morphology, chromosome territories, subchromosomal domains, chromatin loop aggregates, chromatin rosettes, chromatin loops, chromatin fibre, chromatin density, persistence length, spatial distance measurement, histones, H1.0, H2A, H2B, H3, H4, mH2A1.2, DNA sequence, complete sequenced genomes, molecular transport, obstructed diffusion, anomalous diffusion, percolation, long-range correlations, fractal analysis, scaling analysis, exact yard-stick dimension, box-counting dimension, lacunarity dimension, local nuclear dimension, nuclear diffuseness, parallel super computing, grid computing, volunteer computing, Brownian Dynamics, Monte Carlo, fluorescence in situ hybridization, confocal laser scanning microscopy, fluorescence correlation spectroscopy, super resolution microscopy, spatial precision distance microscopy, auto-fluorescent proteins, CFP, GFP, YFP, DsRed, fusion protein, in vivo labelling.

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