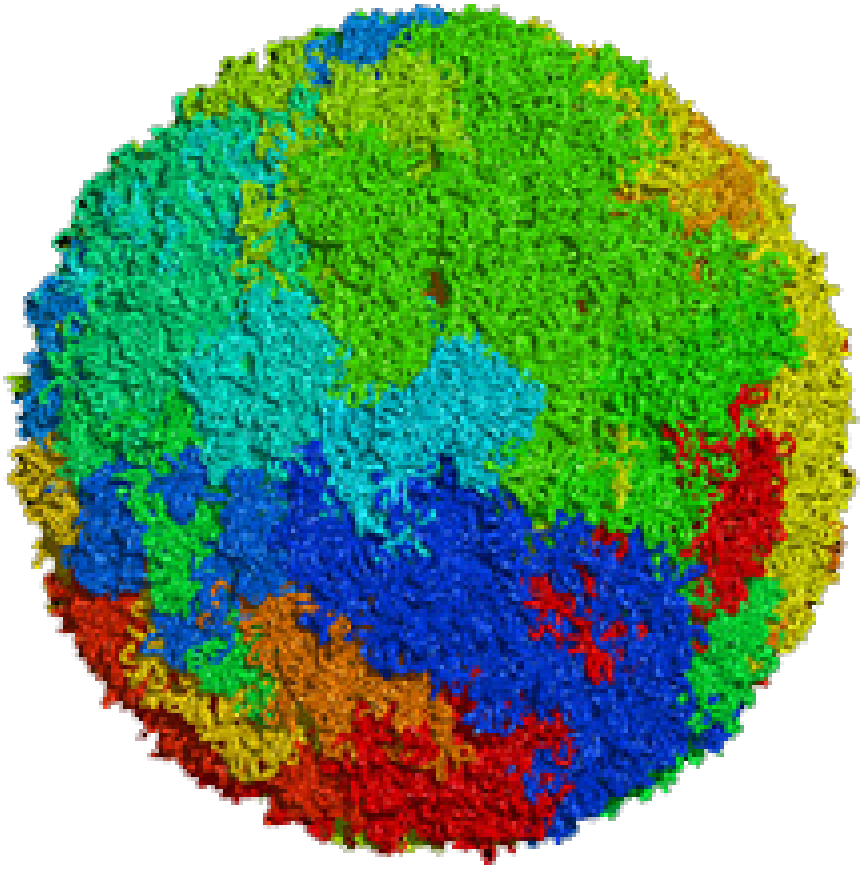
THREE-DIMENSIONAL ORGANIZATION OF CHROMOSOME TERRITORIES AND THE HUMAN INTERPHASE CELL NUCLEUS

SIMULATIONS and EXPERIMENTS



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Biophysics of Macromolecules

German Cancer Research Center (DKFZ)

Heidelberg - Germany

Heidelberg 3D Human Genome Study Group
German Human Genome Project

Typical state of the arts view:

1) **human cell nuclei usually have no spherical shape,**
2) **the DNA is not a closed pipe,**
3) **nucleosomes might not be regularly organized into chromatin,**
4) **chromatin does not float around randomly in the nucleus.**

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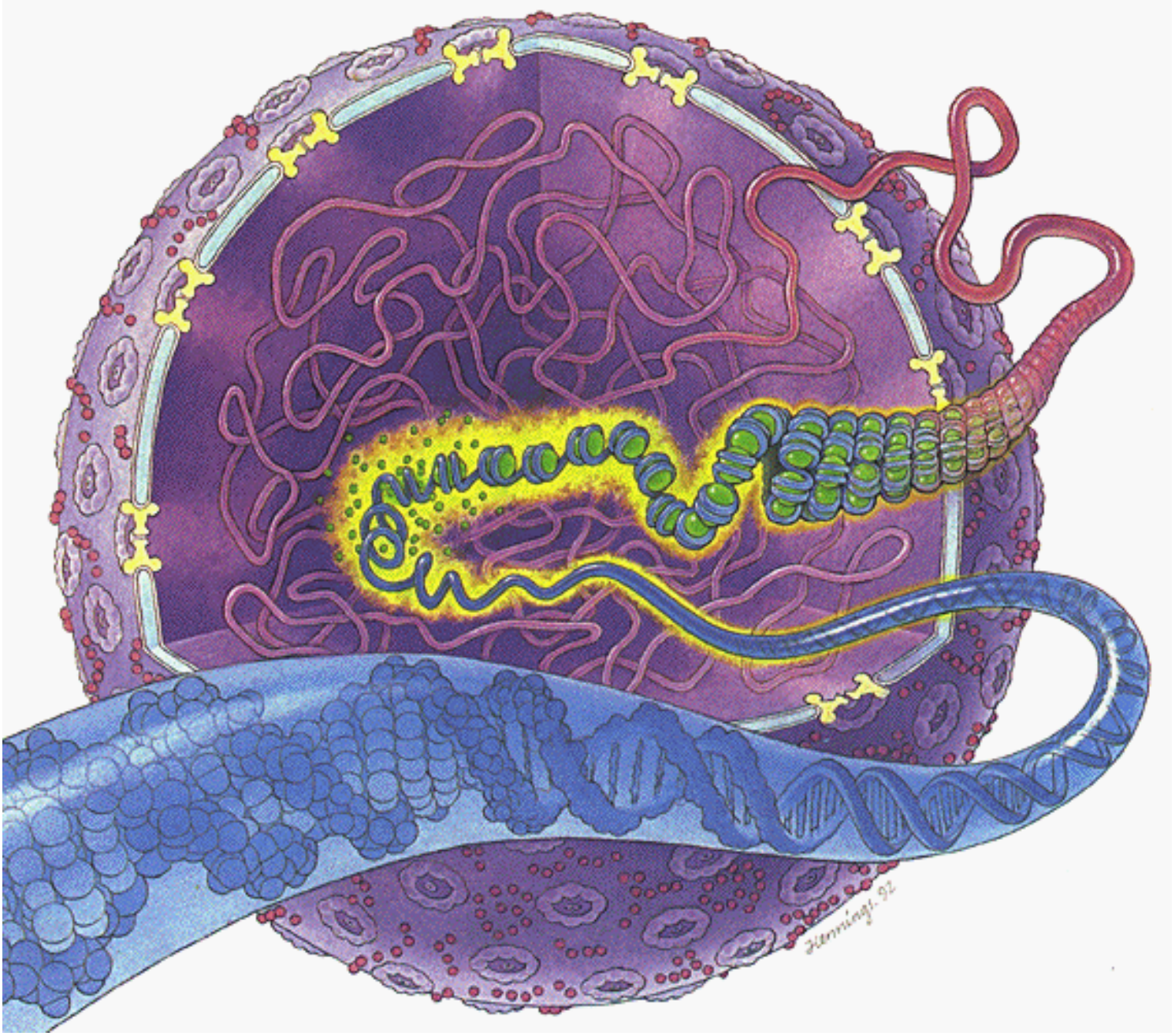

V. Hennings (illustrator) in Molecular and Cellular Biology by Stephen L. Wolfe, 1993.

The dynamic and hierarchical organization of cell nuclei span between 10 and 13 orders of magnitude concerning length and time scales.

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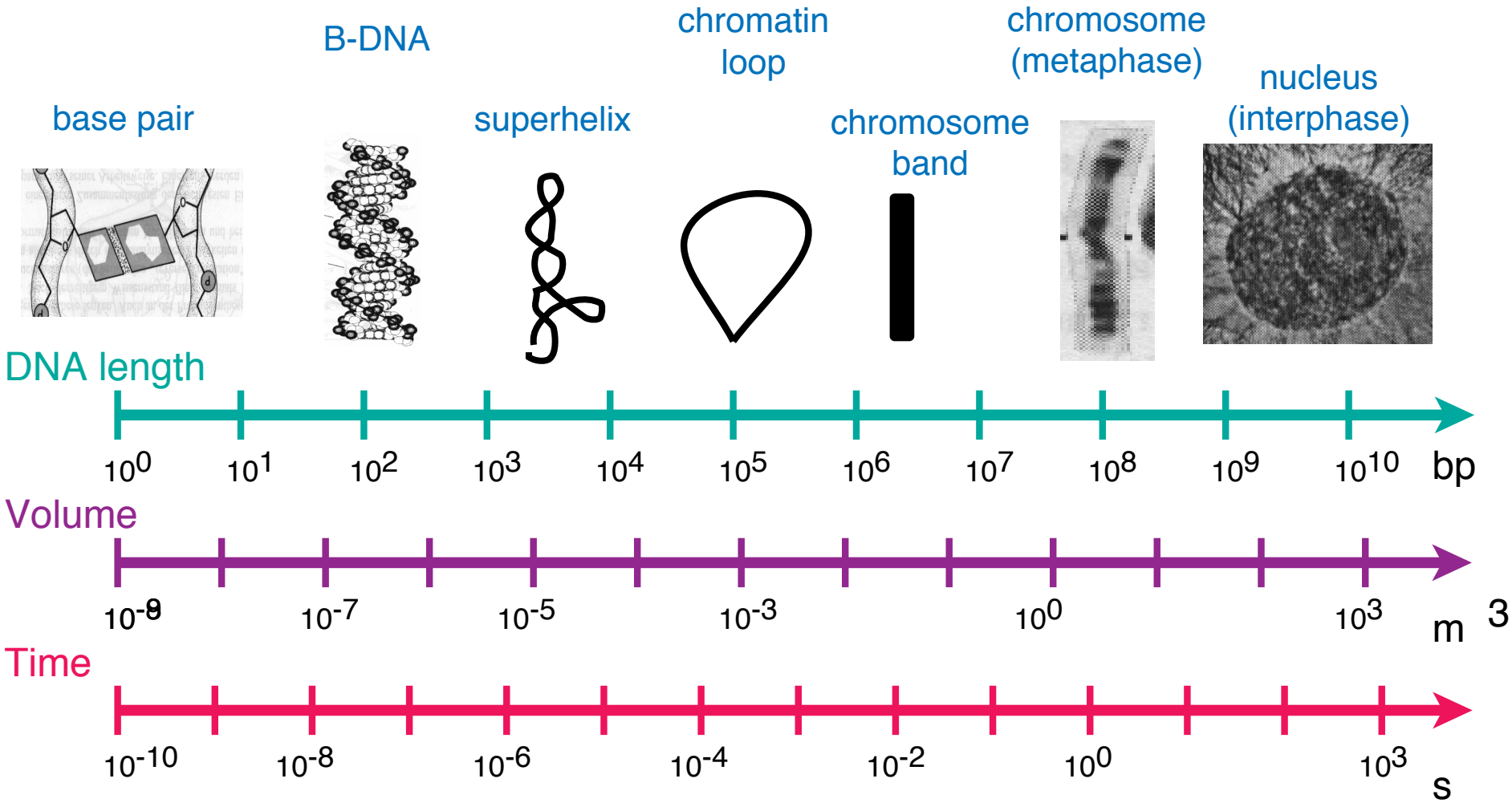
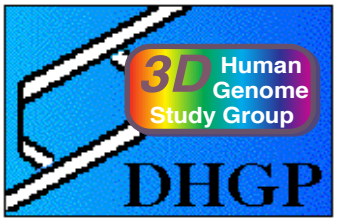


Overview



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Experiment

Prader-Labhard-Willi/ Angelmann Region

↓

fluorescence in-situ hybridization (FISH)

↓

3D confocal scanning microscopy

Simulation

Multi-Loop-Subcompartment and Random Walk/ Giant Loop model

↓

polymer model for simulation of the chromatin fiber

↘ ↙

Conclusions for the human cell nucleus

chromosome-, chromosome-arm and subcompartment overlap

3D-distances between genomic markers as function of their genomic separation

behaviour of marker ensembles and dynamics of structural features

fractal properties of chromosomes

decondensation of chromosomes from metaphase into interphase and chromosome stretching

conclusions from simulating whole cell nuclei

Fluorescence in-situ Hybridization

FISH

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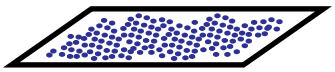
Cell - Preparation

cells on coverslip grown to confluent layer

fixation of cells on coverslip (formaldehyde) and permeabilisation



DNA double strand

TACGTTAACGGTAGCATT
ATGCAATTGCCATCGTAA

Probe - Preparation

finding of genomic site for marking and cloning of this sequence

AACGG
TTGCC

labeling of the DNA probe (Nick translation or PCR) with

Digoxigenin (indirect)

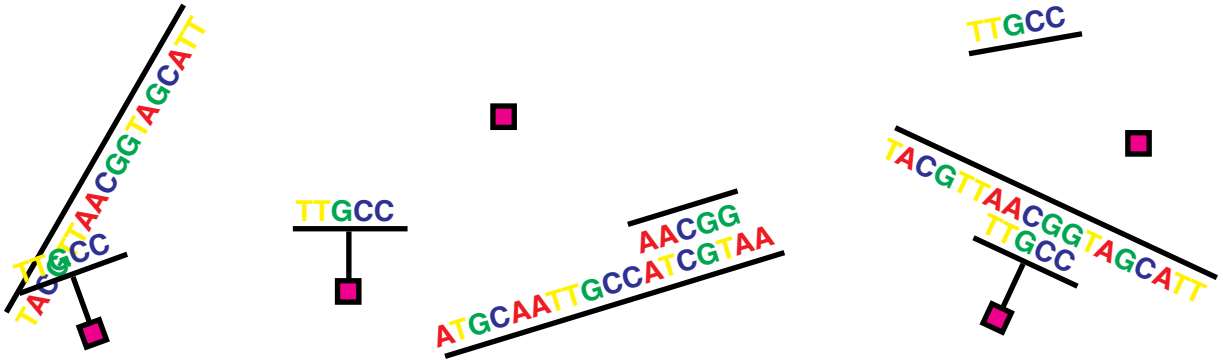
AACGG
TTGCC

Fluorophor (direct)

AACGG
TTGCC

Hybridization

probe is put on coverslip and melting of the double strands at 70C



amplification with fluorescent labeled antibodies

TACGTTAACGGTAGCATT
TTGCC

Ideogram of chromosome 15 with
Prader - Willi Region and Angelmann Region.
The size and genomic distance of the clones
are sufficiently small and well characterized to measure
the fine structure and organization of chromosome territories.

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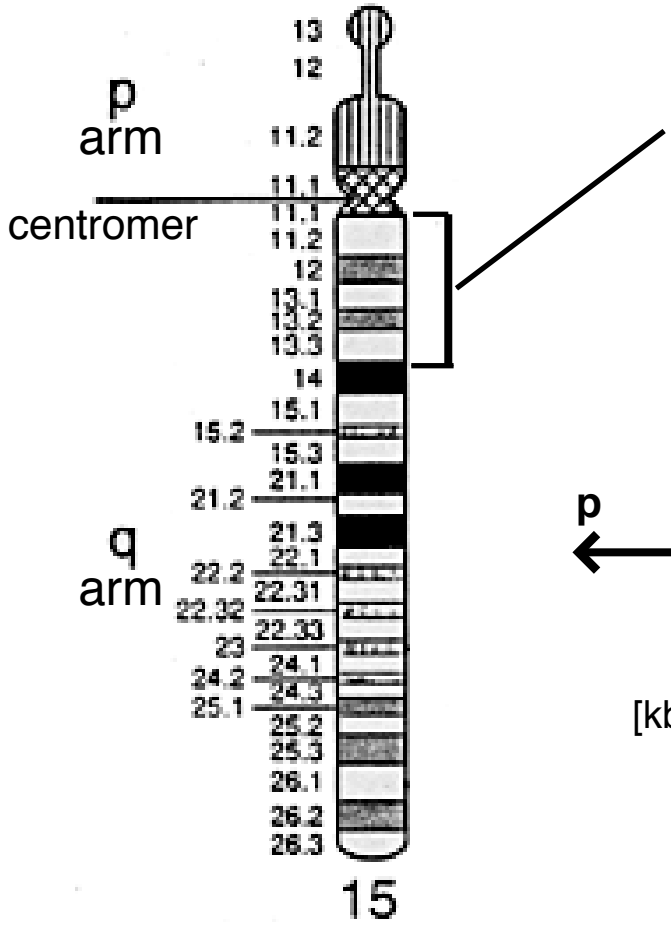
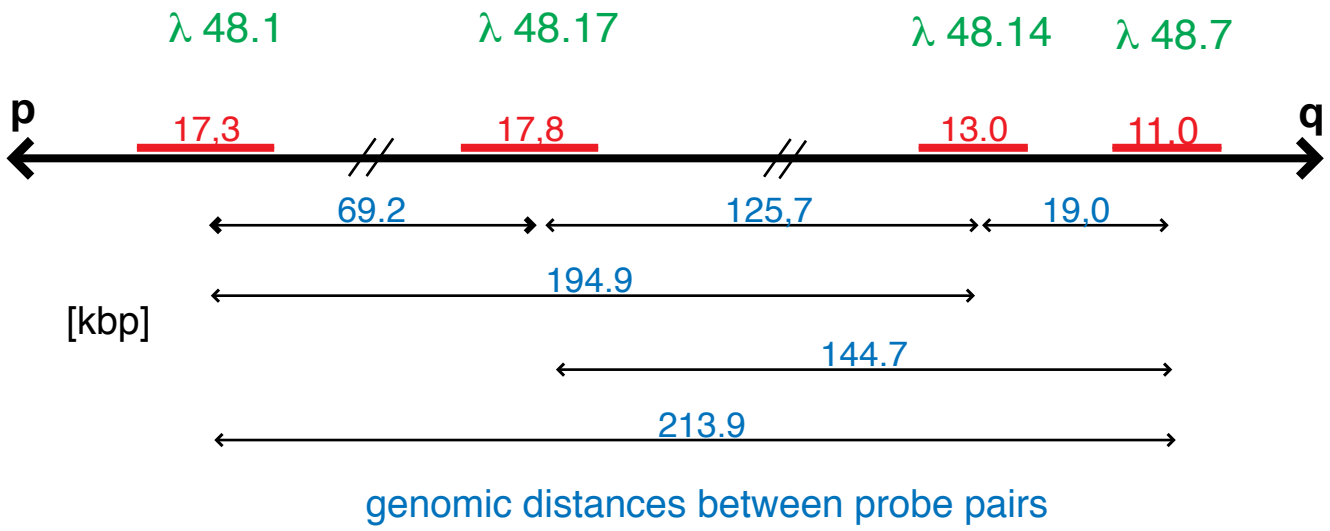


Prader-Willi Region (paternal chromosome) **YAC 48**
Angelmann Region (maternal chromosome) **YAC 60**

↕ 1Mbp

clones and their genomic size



genomic distances between probe pairs

Principle of the Confocal Laser Scanning Microscope and Leica TCS NT setup.

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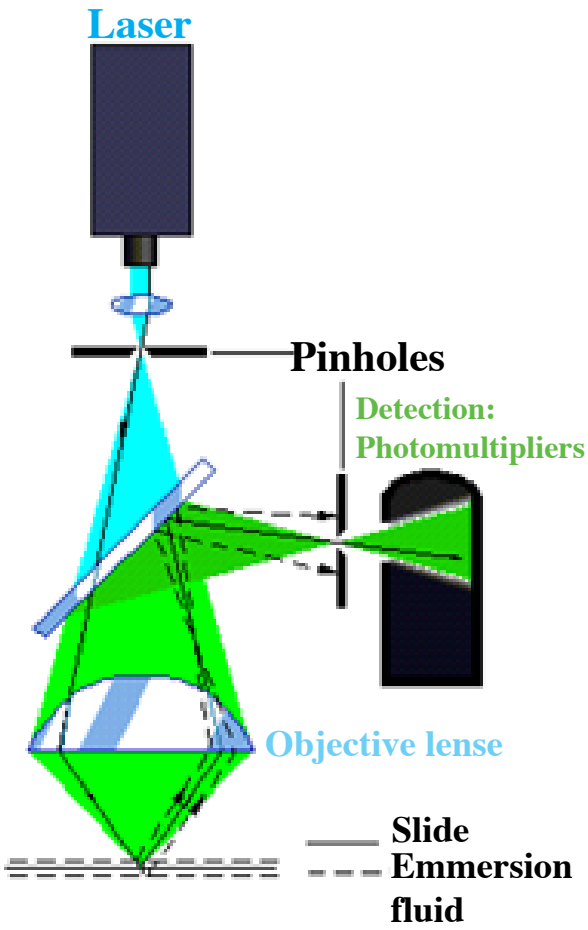

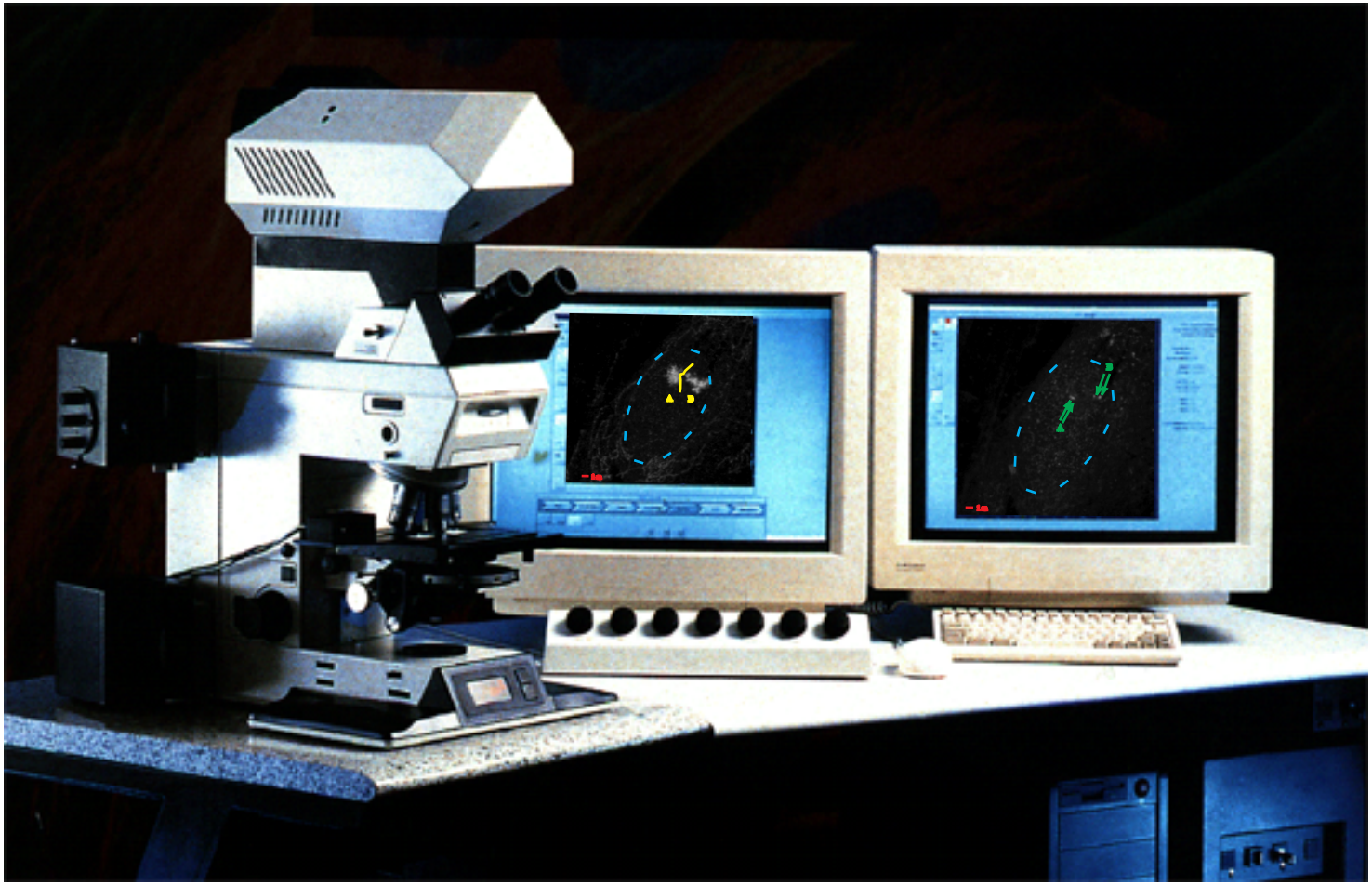

Chromosomes form distinct territories in interphase and genomic markers lie within the territories and are clearly separable.

Left: Territory painting by FISH of chromosome 15; by chance the two territories neighbour each other.

Right: Genomic markers YAC48 and YAC60, genomic separation 1 Mbp.

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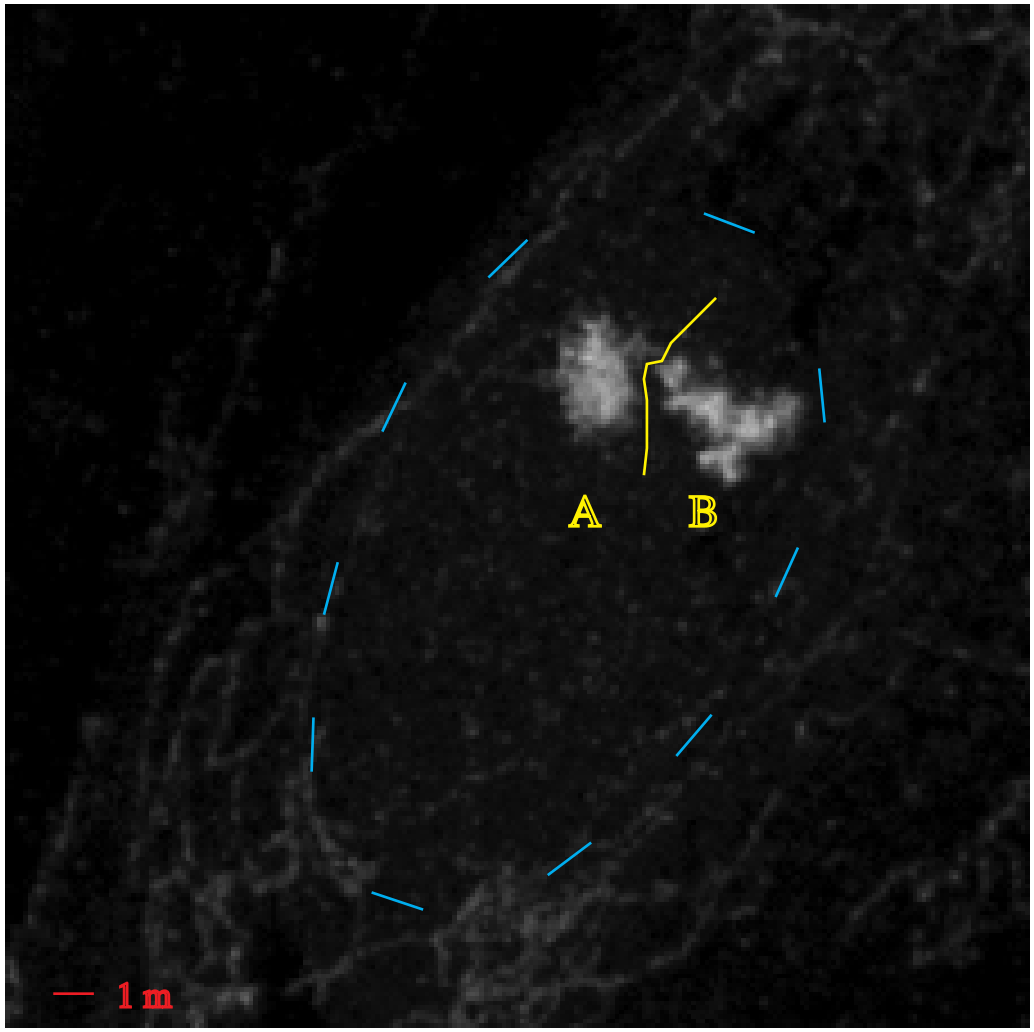

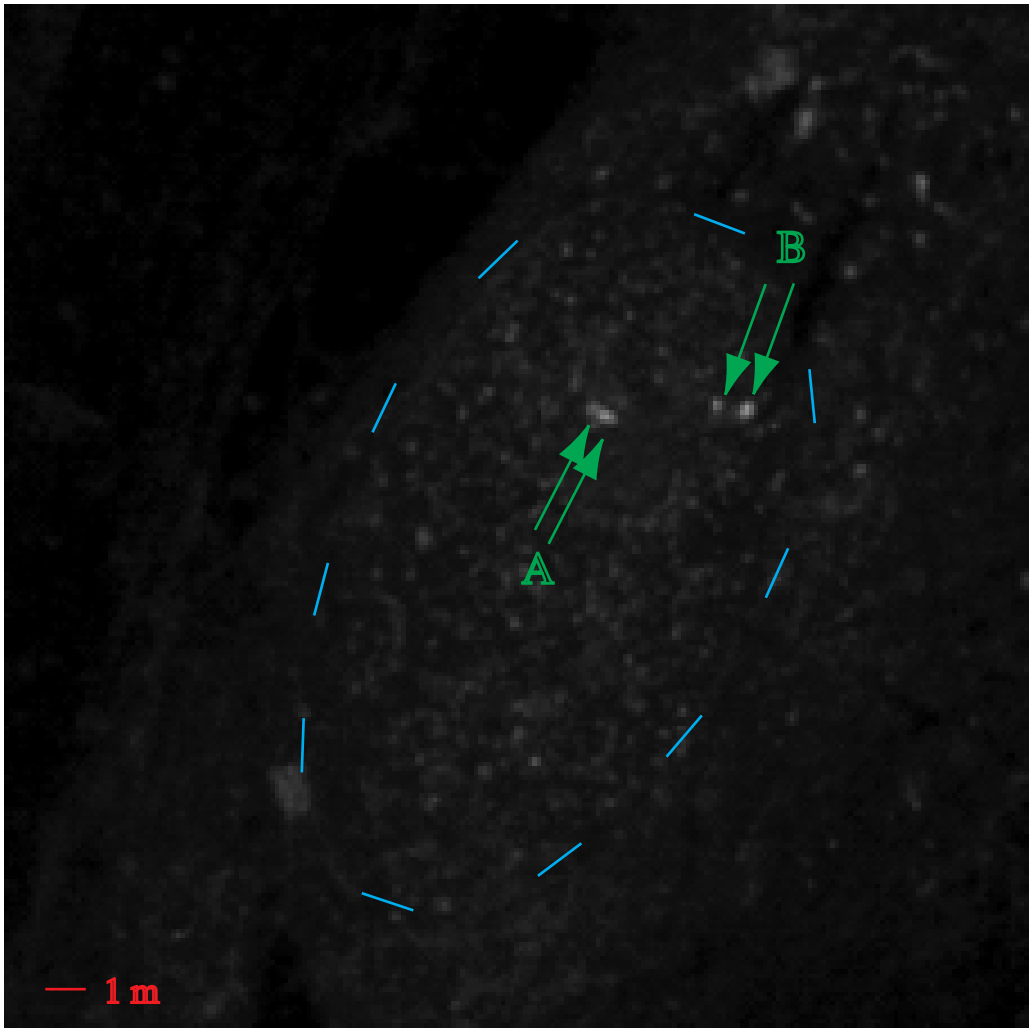

Dual colour FISH of genomic markers leads to measurements of 3D-distances which are below the resolution of the microscope. Critical signals could also be excluded with higher confidence.

Genomic marker λ48.1 in red and marker λ48.14 in green, genomic separation 195 kbp.

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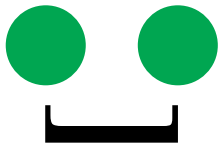
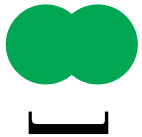
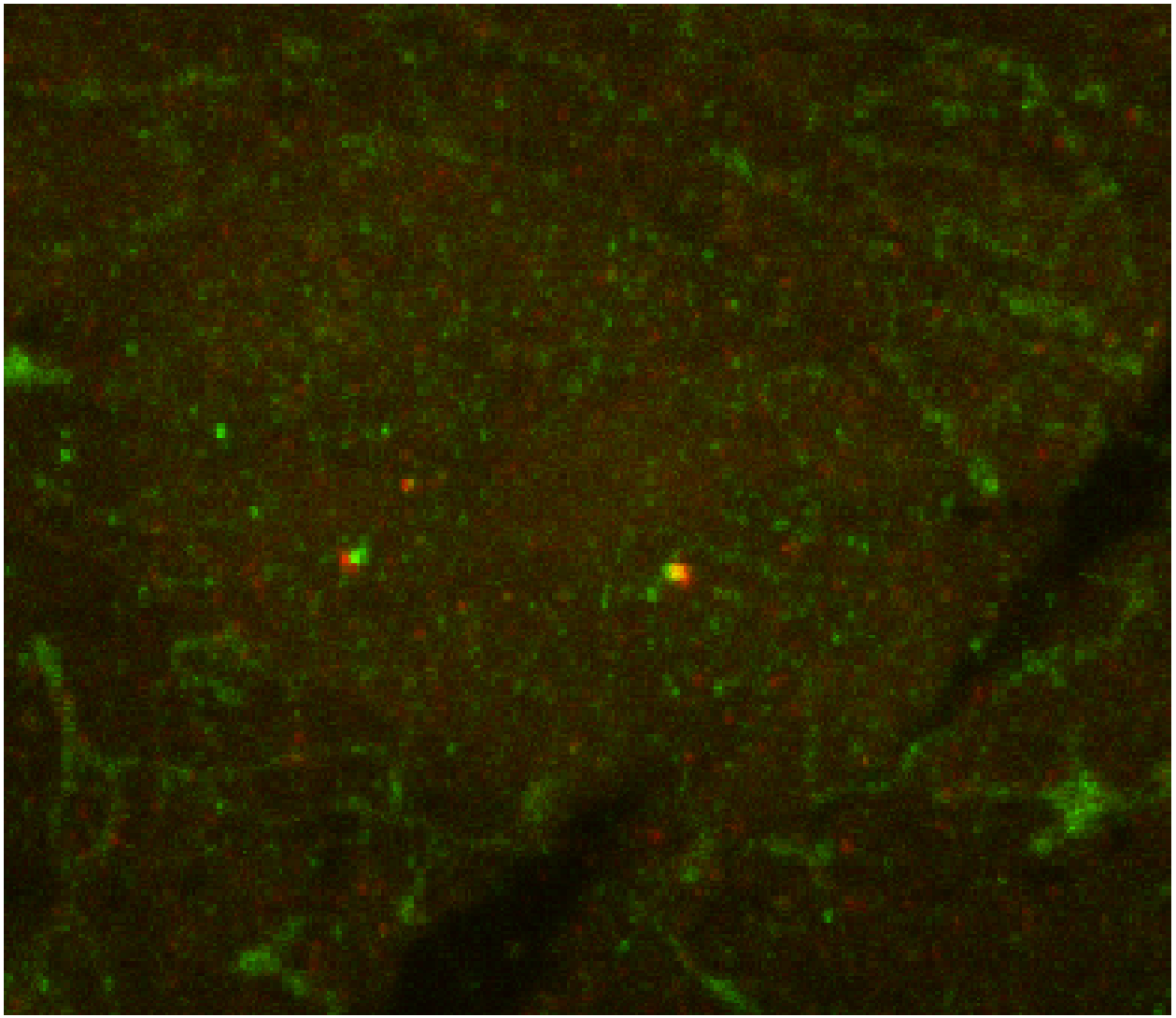
one colour



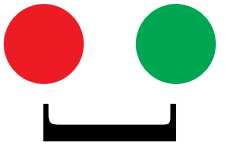
d



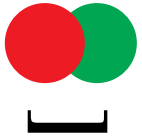
d ?



dual colour



d



d

Statistical analysis of the spatial distances between the PWS-Region (YAC48) and AS-Region (YAC60) with a genomic distance of 1Mbp = 10m chromatin fiber.

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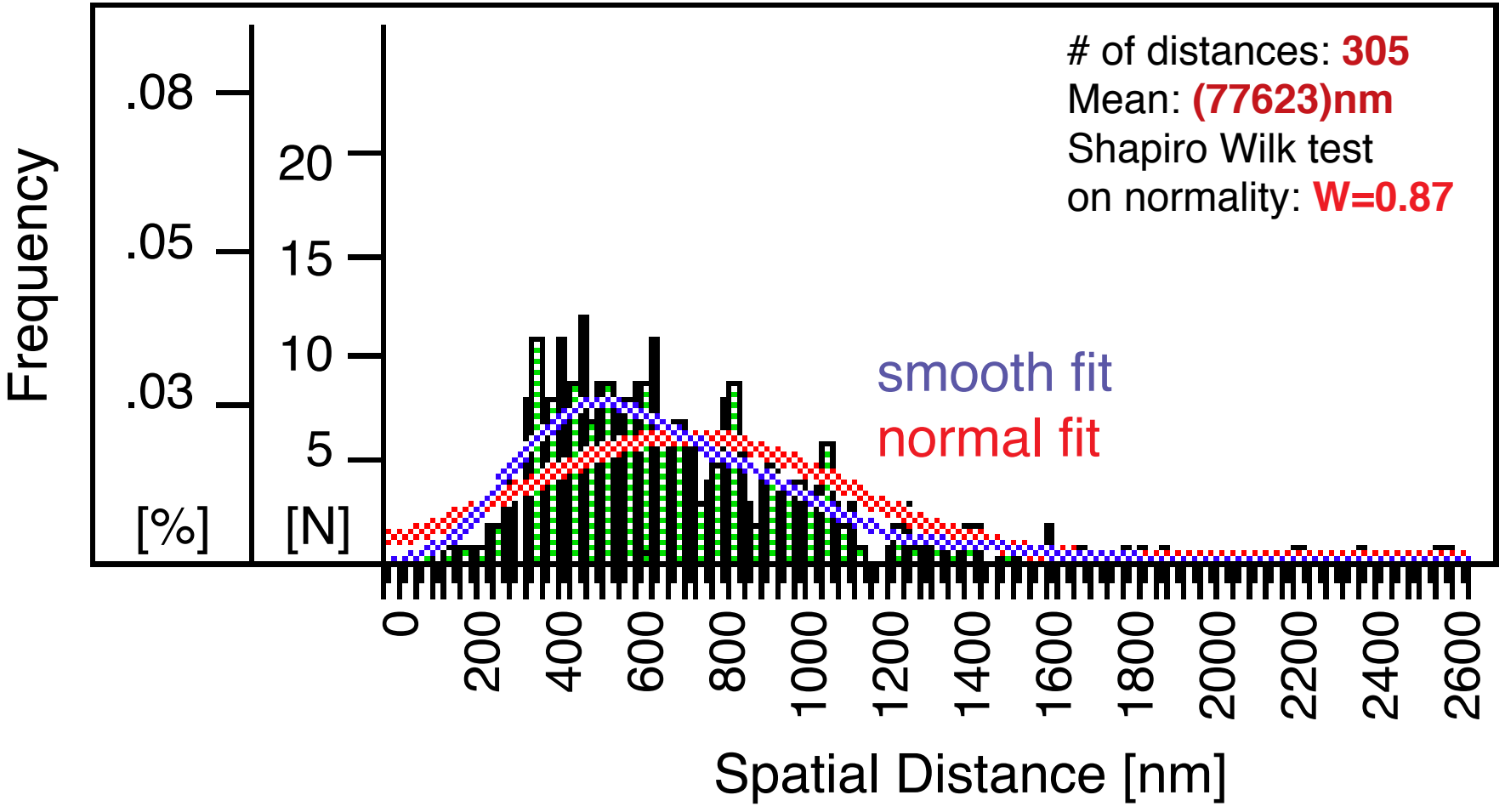
Distance Distribution



Multi-Loop-Subcompartment Model versus Random Walk / Giant Loop Model. Rosettes in the MLS-Model correspond to the size of chromosomal interphase band domains.

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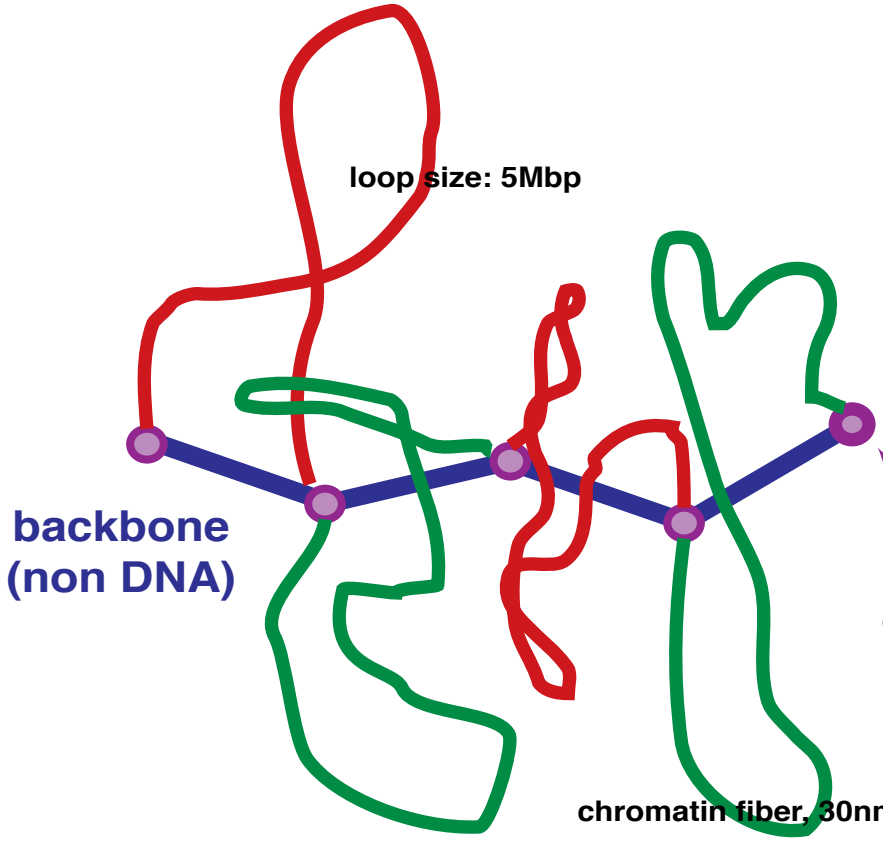
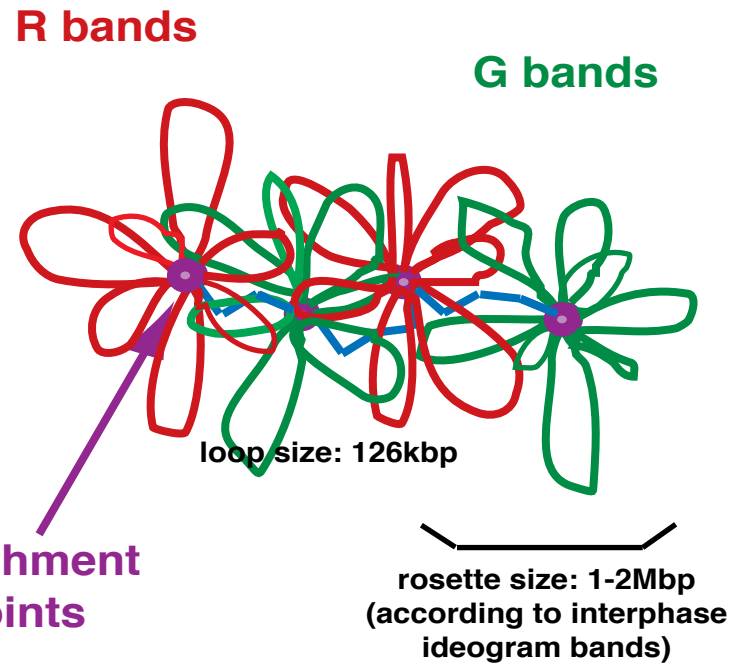
Random Walk / Giant Loop model (RW/GL) Sachs et al. (1995)



Multi-Loop-Subcompartment model (MLS) Münkel et al. (1997)



Linker consists of DNA
(in contrast to backbone)

Polymer Chain and Potentails

The chromosome fiber is simulated assuming a polymer chain and harmonic potentials.

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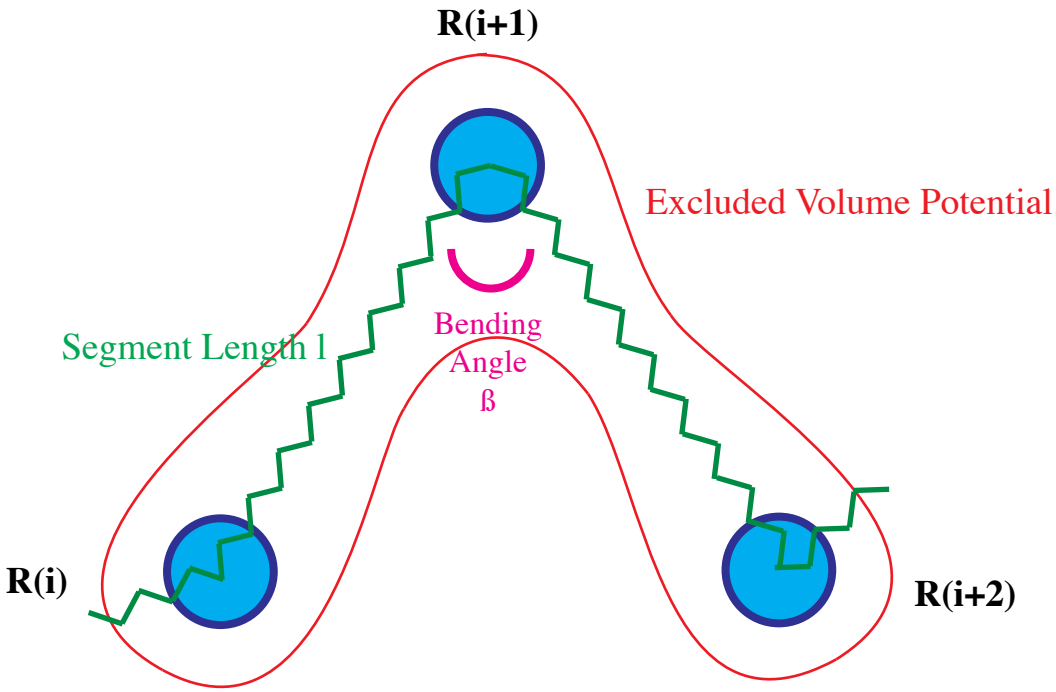

Stretching Potential

$$U_s(l) = \frac{k_B T}{2^2}(l - l_0)$$

Bending Potential

$$U_b(ß) = \frac{k_B T}{2^2} ß^2$$

Excluded Volume Potential

$$U_{ev}(r) = U_{ev}^0 k_B T \left(1 + \frac{r^4 - 2r_c^2 r^2}{r_c^4}\right)$$

k_B : Bolzmann constant
T : Temperature, 310 K
: stretching elasticity
: bending elasticity
r_c : minimum distance of segments

Random-Walk/Giant-Loop model versus Multi-Loop-Subcompartment model. Simulation results of chromosome 15.

The chromosome is simulated assuming a flexible polymer chain, starting with ~ 3500 300nm=31kbp and relaxing with ~ 21,000 50nm=5.2kbp segments. The starting configuration has the approximate form and size as in metaphase. 50 parallel simulations and their evalutation take 5.5 years single CPU-time.

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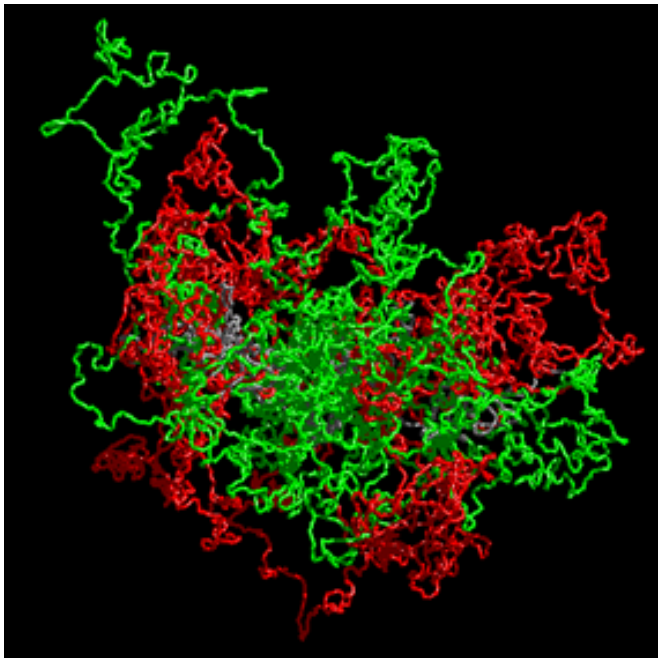
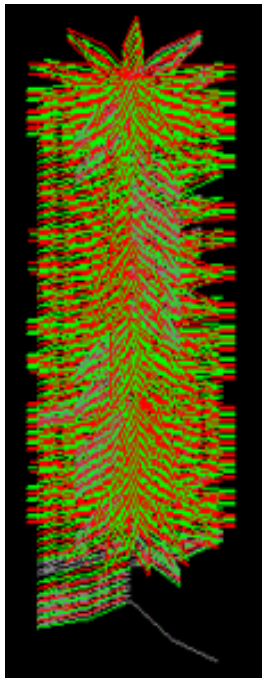
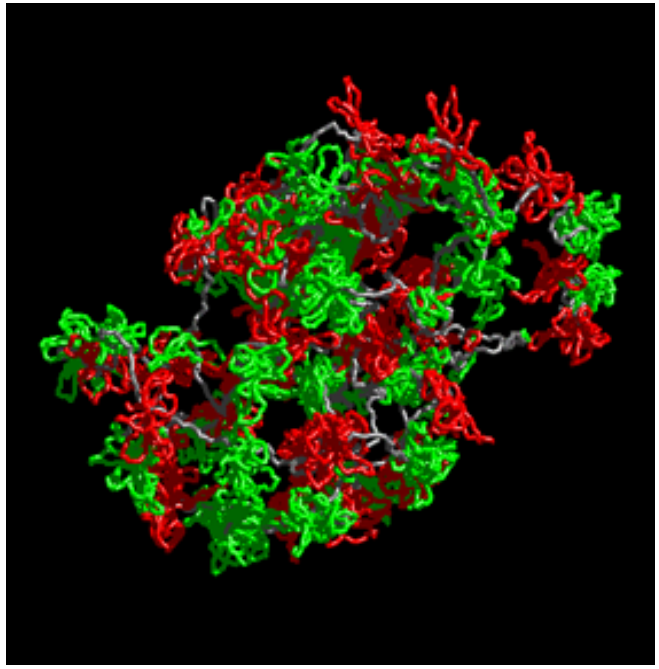
Ray traced image of the Random-Walk/Giant-Loop model, loop size 5Mbp, after ~80.000 Monte-Carlo and 1000 relaxing Brownian-Dynamics steps. Large loops intermingle freely thus forming no distinct features like in MLS model.





Wire frame image of the metaphase chromosome resembling starting configuration.

Ray traced image of the Multi-Loop-Subcompartment model, loop size 126kbp, linker size 126 kbp, after ~50.000 Monte-Carlo and 1000 relaxing Brownian-Dynamics steps. Here rosettes form subcompartments as separated organizational and dynamic entities.



The MLS-model leads to low overlap of chromosome-arms and subcompartments in contrast to the RWGL-model. This is also seen in experiments.

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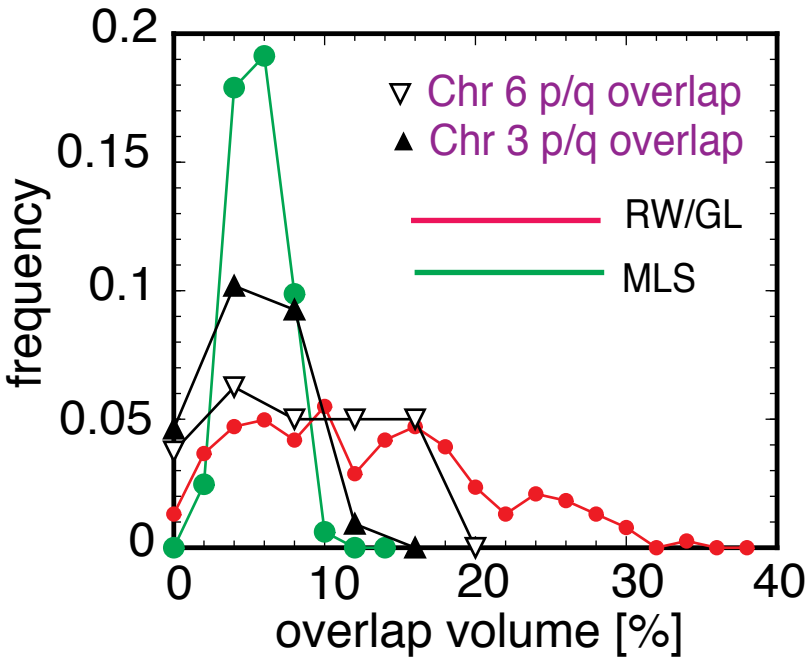
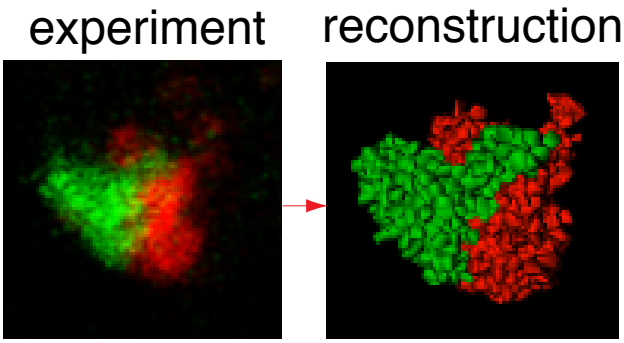
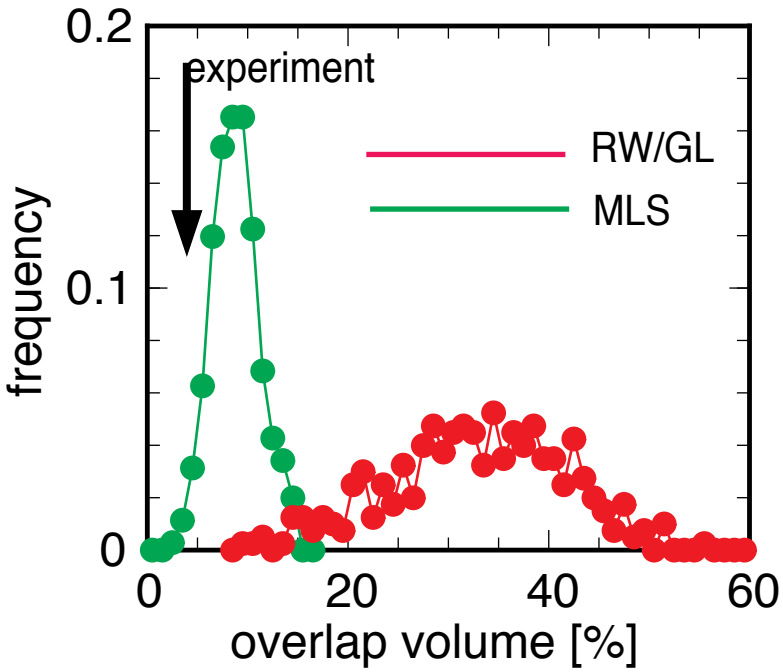
Arm - Overlap



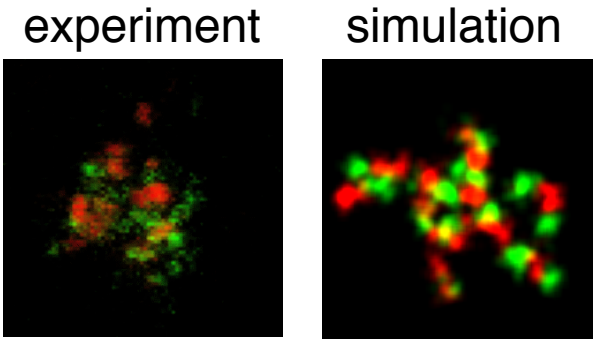
Confocal images of interphase p- and q- arms of human chromosome 3



Subcompartment - Overlap



Confocal images of interphase R- and G- bands of human chromosome 15



Random-Walk / Giant-Loop versus Multi-Loop-Subcompartment model.

Best agreement between simulations and experiments is reached for a Multi-Loop-Subcompartment model with a loop size of 126kbp and a linker length of 126kbp.

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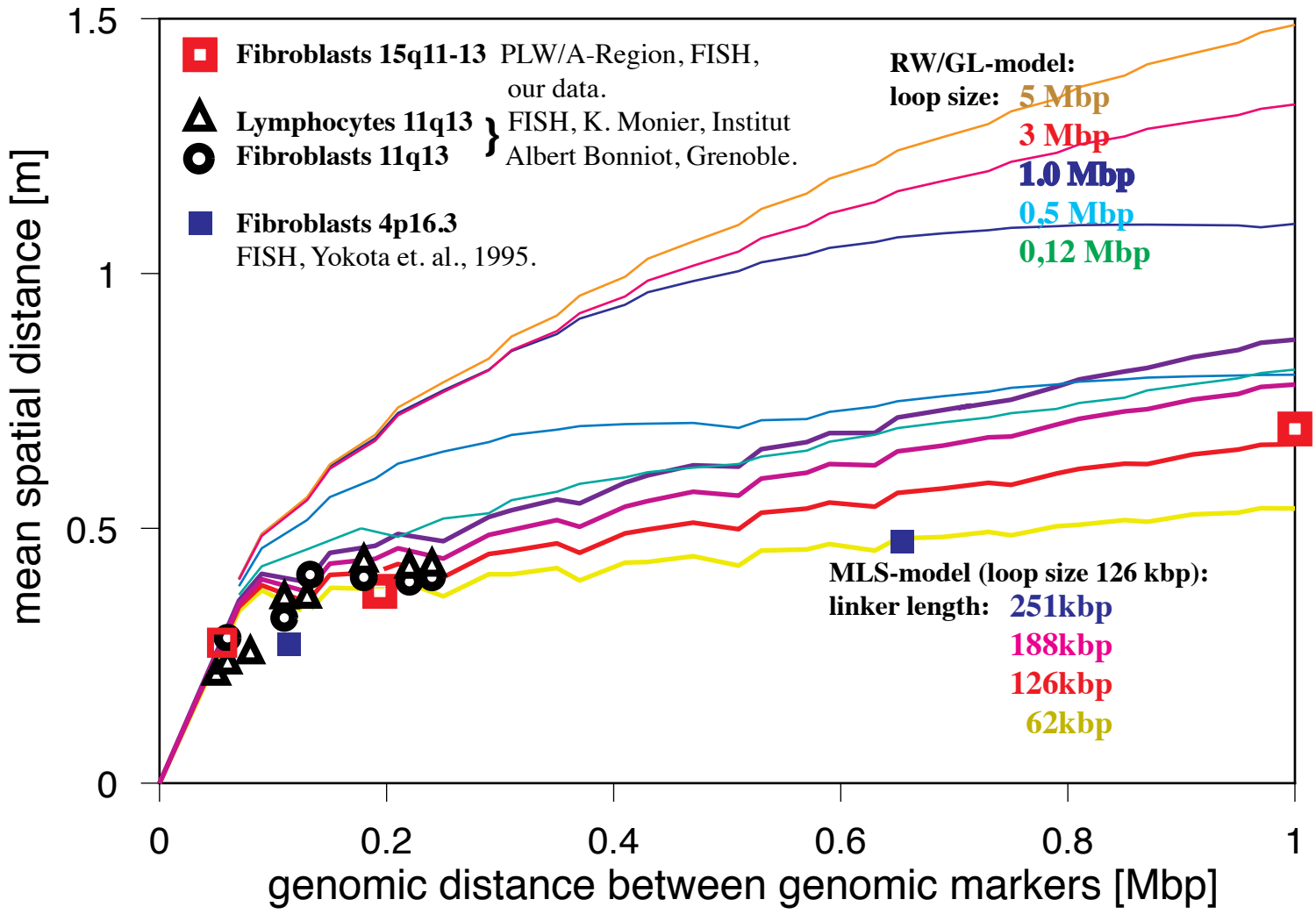

Shift of a marker ensemble through a rosette in the MLS-model in respect to loop bases.
This leads to different sets of 3D-distances for every ensemble position.
Due to the symmetry of the MLS-rosettes periodicities are found.

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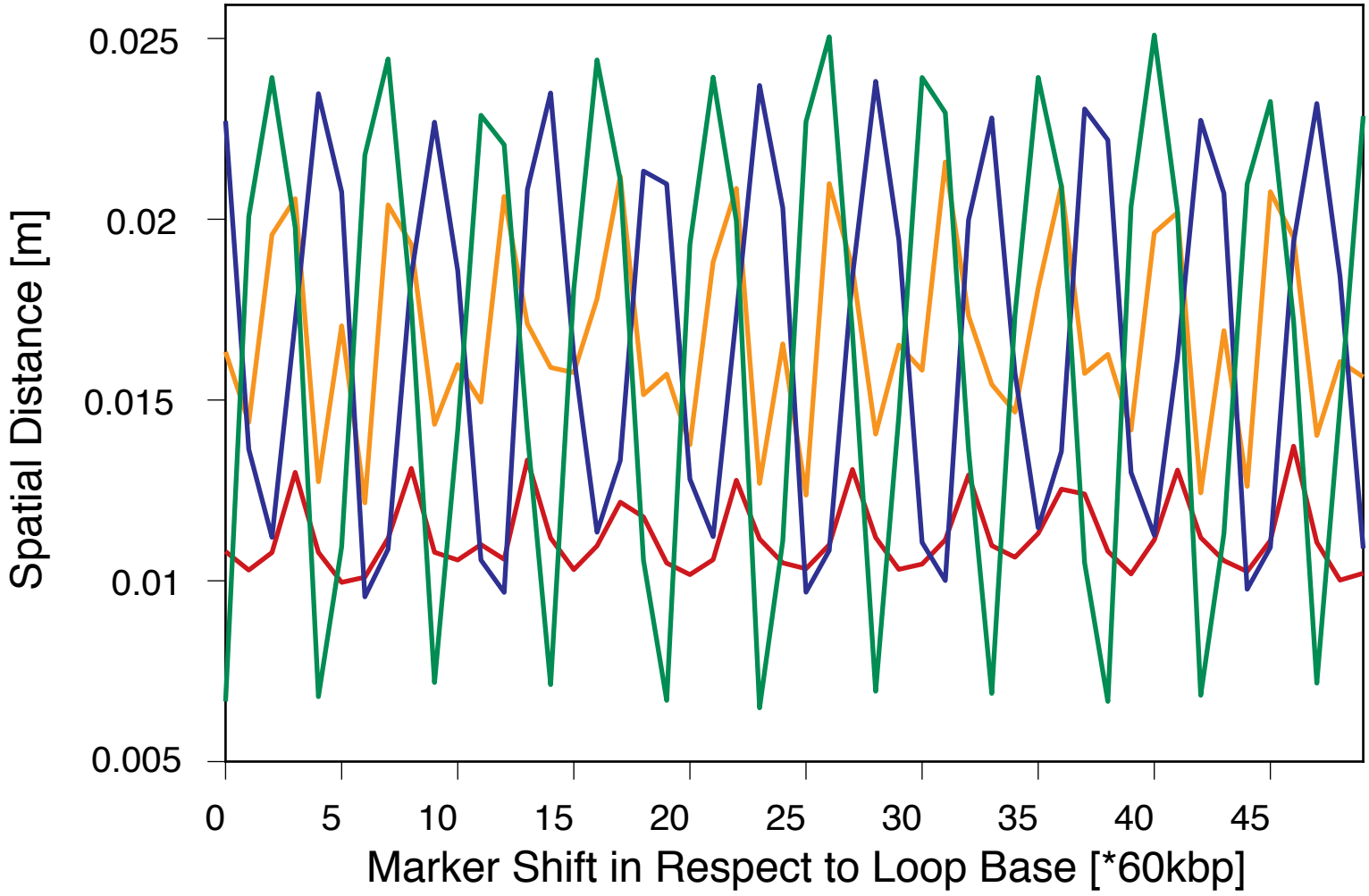

In agreement with porous network research fractal analysis show multifractal behaviour in simulations of chromosome 15. Different fractal dimensions mean different process-dynamics in these spaces. Therefore chromosomal territories show a higher degree of determinism than previously assumed.

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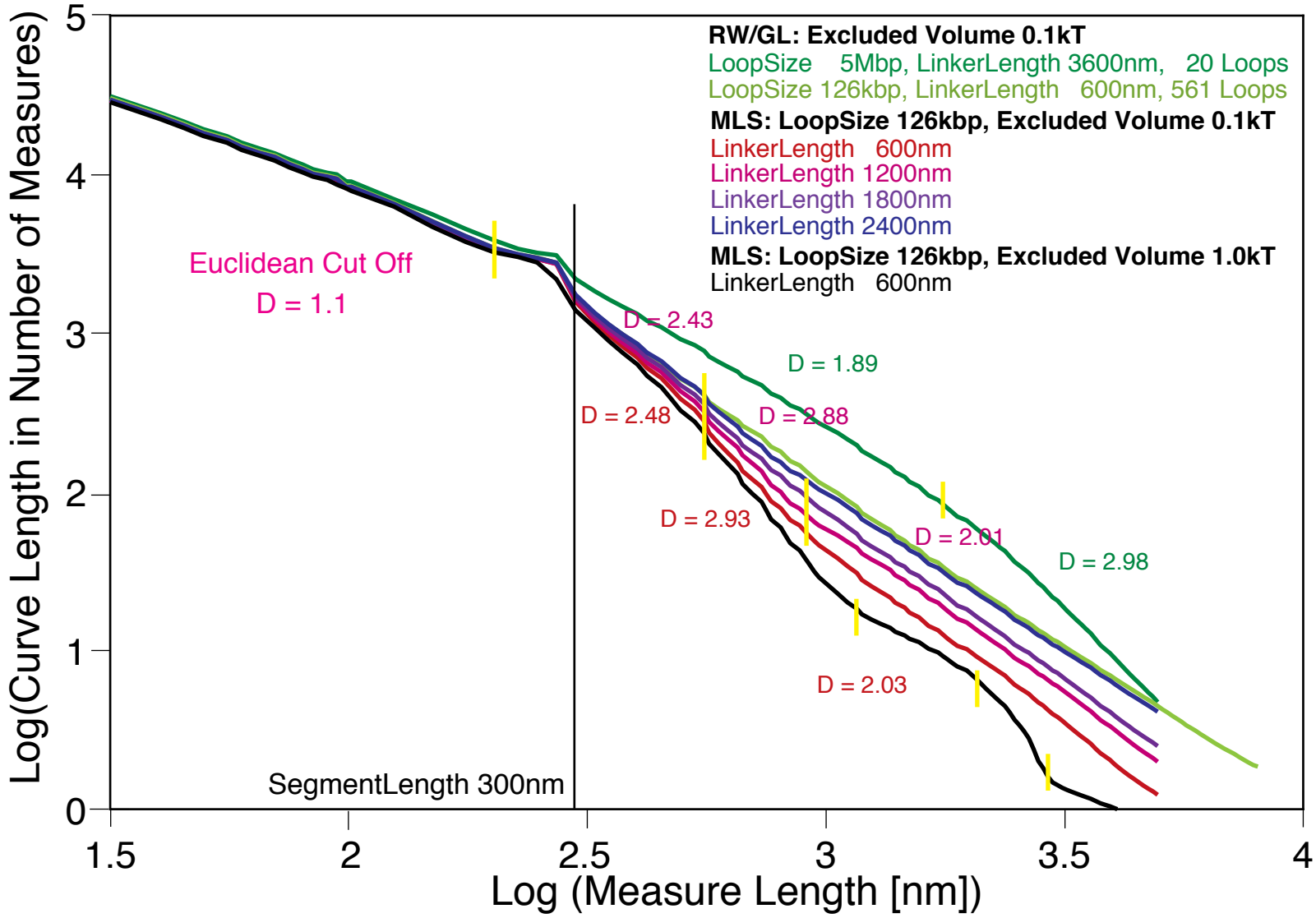

Simulation of Chromosomal Elasticity

Visualization with "Virtual Microscope" of chromosome 15 (MLS model, 8 subcompartments) under external stress. Subcompartments are shown as a projection image of a confocal laser scanning microscope image series.
left: external force = 0 fN
right: external force = 1.2 fN

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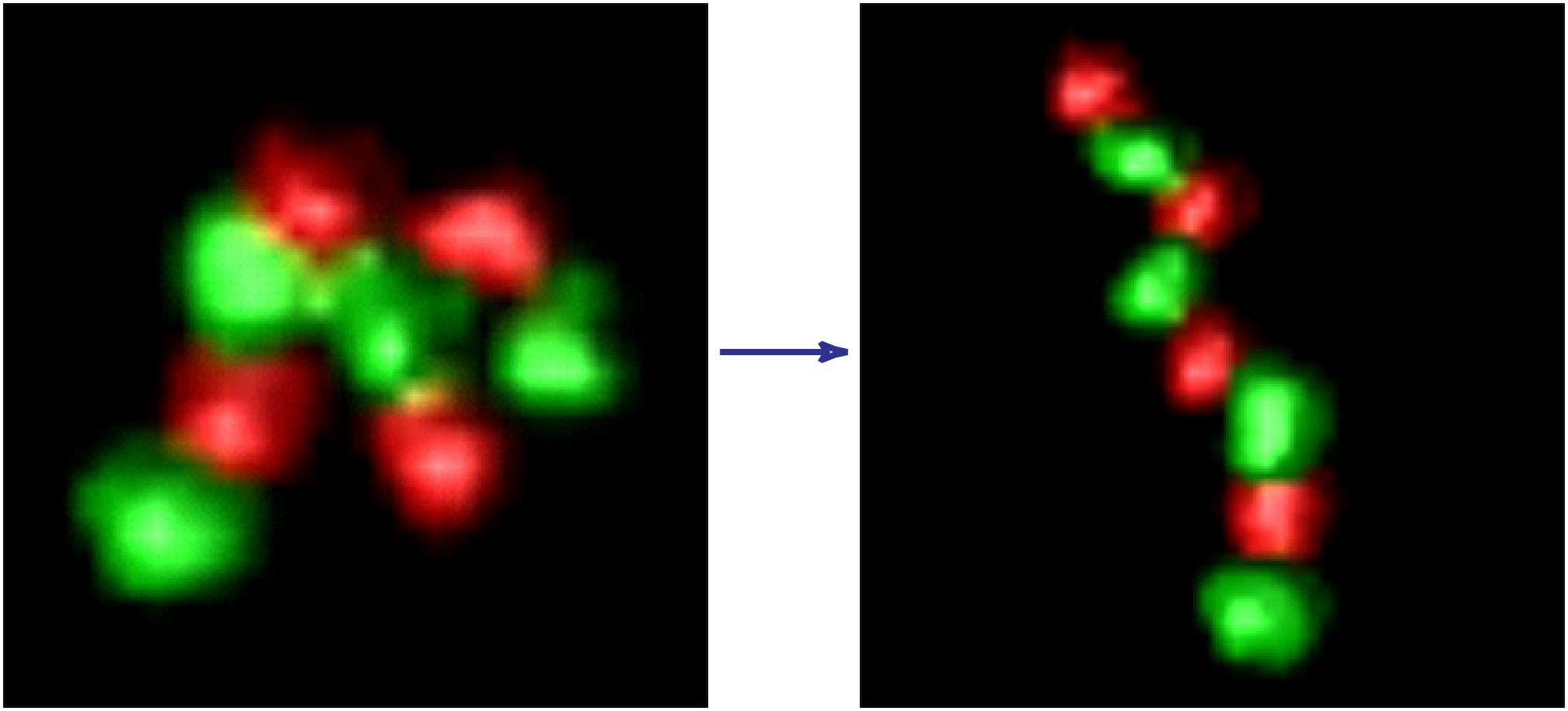

Simulation of Chromosome Elasticity

Force strain curve of an interphase
Multi-Loop-Subcompartment-model (MLS) for chromosome 15.
Young´s modulus for external forces below 5 femtonewtons (fN): (3,00,4) fN.

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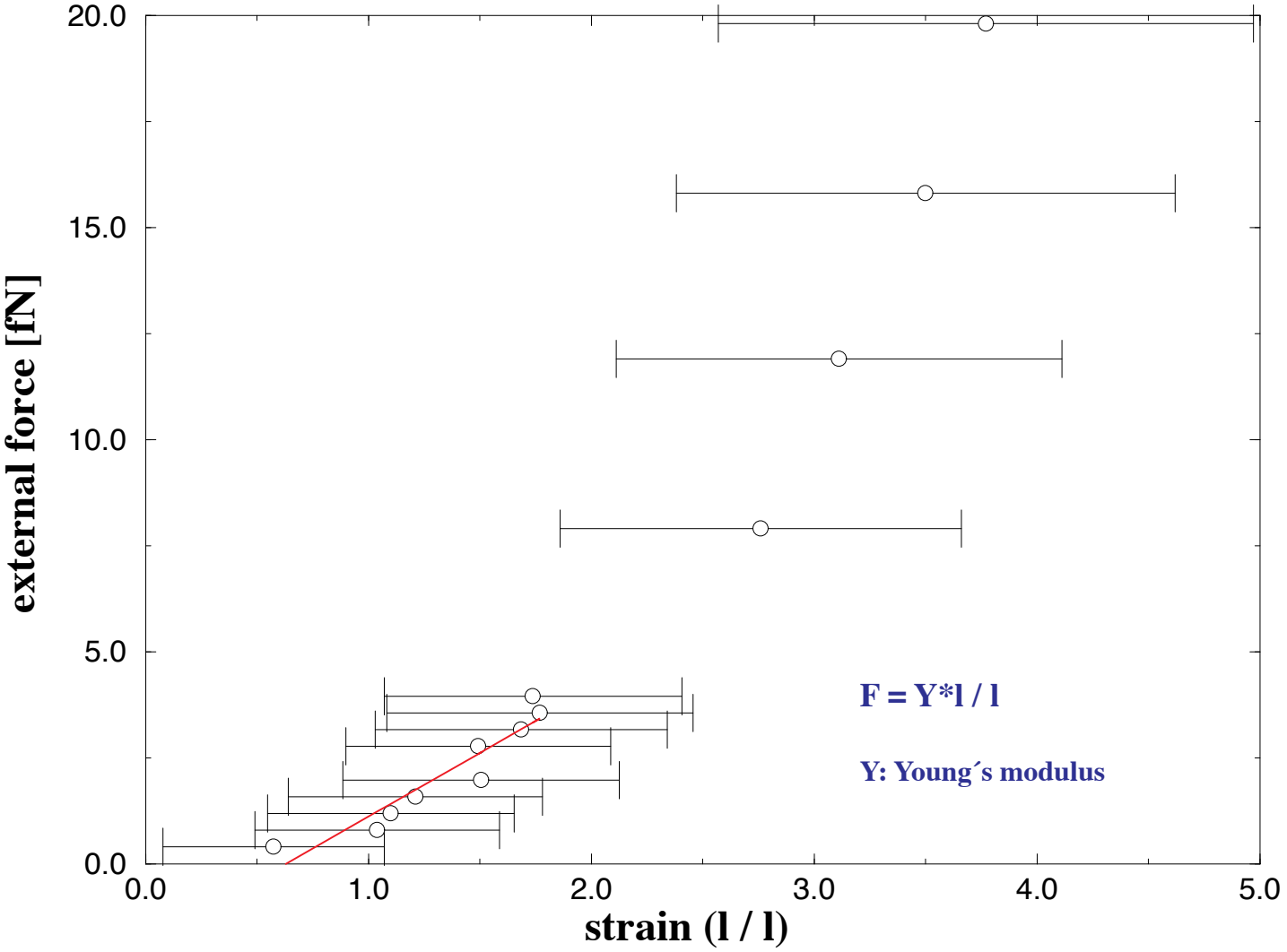

´Virtual Human Cell Nucleus´

Simulation of all 46 chromosomes using the Multi-Loop-Subcompartment model.

The nucleus is simulated assuming a flexible polymer chain, modelling the 46 chromatin fibers with in total 1,248,794 50 nm = 5.2 kbp segments. Pictures are shown after a 0.5 ms Brownian Dynamics simulation, one step taking 10s. As starting configuration a metaphase nucleus was chosen.

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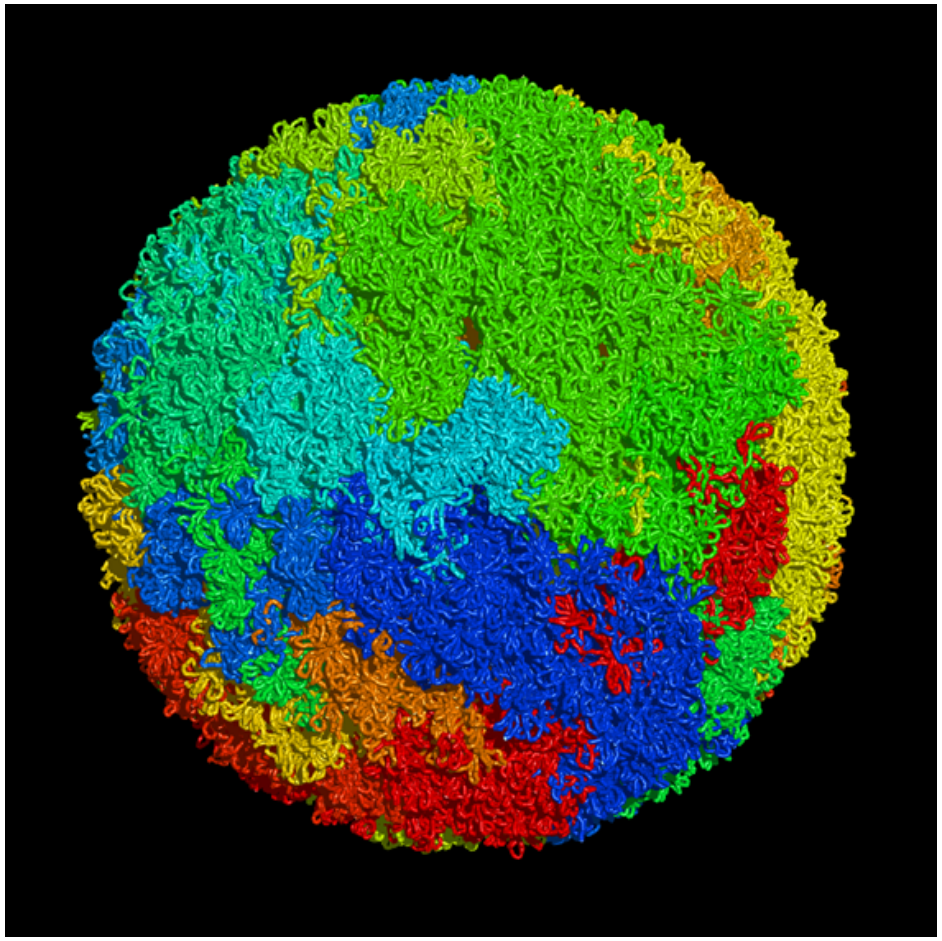
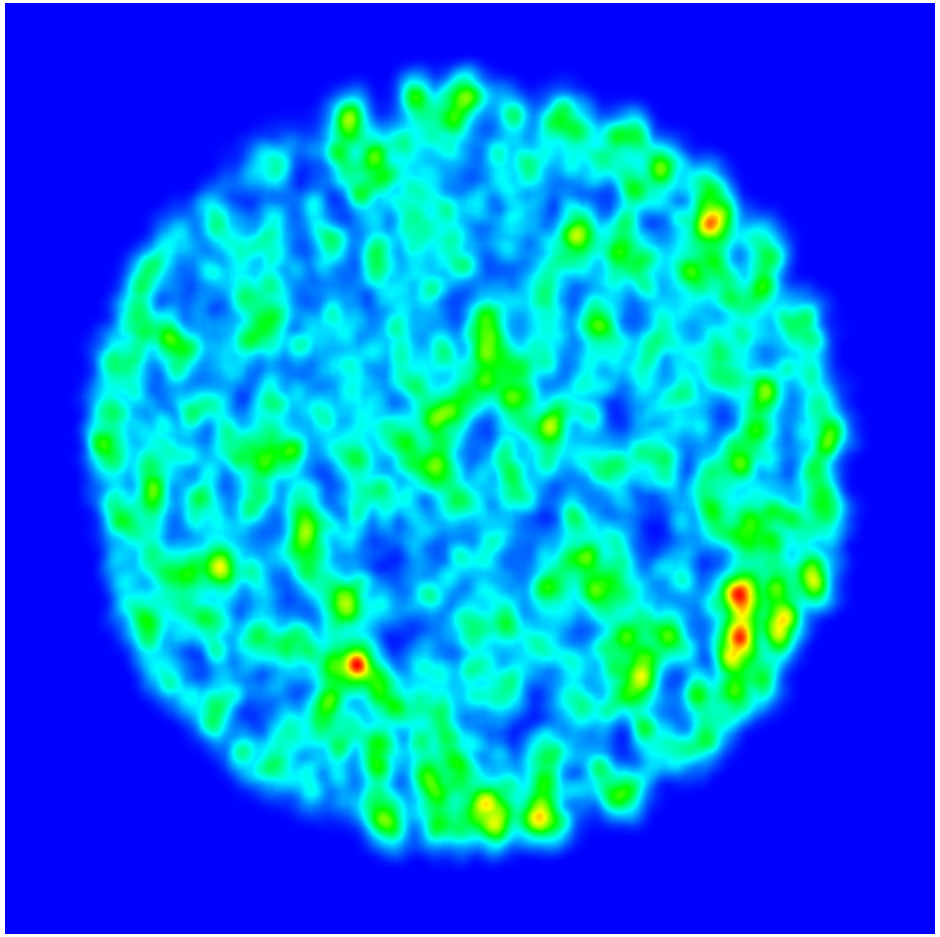
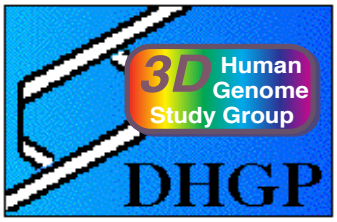


3-D rendering



simulated confocal section

Conclusions



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Best agreement between simulations and experiments is reached for a Multi-Loop-Subcompartment-model with a loop and linker size of 126 kbp (1200nm).

Supposed that defined loop bases exist it might be possible to determine the positioning of genes relative to each other.

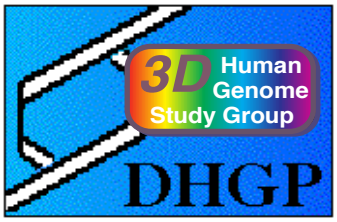
Chromosomes show multifractal behaviour in good agreement with predictions drawn from porous network research.

Chromosome decondensation and stretching lead to comparable results from experiments.

Simulations of whole cell nuclei lead to the formation of distinct chromosome territories.

The Multi-Loop-Subcompartment-model leads to low overlap of chromosome territories, chromosome arms and chromosome subcompartments in contrast to the RandomWalk/Giant Loop-model.

People



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Harald Bornfleth
Christoph Cremer
Institute for Applied Physics, University of Heidelberg, Germany

IBM-SP2 with 80 nodes, German Cancer Research Centre, Heidelberg
IBM-SP2 with 512 nodes, Computing Centre, Karlsruhe
Silicon Graphics-Graphic-Lab, Institute for Scientific Computing (IWR), Heidelberg

The work is part of the Heidelberg 3D Human Genome Study Group which is part of the German Human Genome Project.

We would like to thank the German Ministry for Sience and Technology (BMFT) for financing this project.

Three-Dimensional Organization of Chromosome Territories in the Human Interphase Nucleus

Knoch, T. A., Münkel, C. & Langowski, J.

Supercomputing at the German Cancer Research Centre (DKFZ), *German Cancer Research Centre (DKFZ)*, Heidelberg, Germany, 2nd July 1998.

Abstract

The synthesis of proteins, maintenance of structure and duplication of the eukaryotic cell itself are all fine-tuned biochemical processes that depend on the precise structural arrangement of the cellular components. The regulation of genes – their transcription and replication - has been shown to be connected closely to the three-dimensional organization of the genome in the cell nucleus. Despite the successful linear sequencing of the human genome its three-dimensional structure is widely unknown.

The nucleus of the cell has for a long time been viewed as a 'spaghetti soup' of DNA bound to various proteins without much internal structure, except during cell division when chromosomes are condensed into separate entities. Only recently has it become apparent that chromosomes occupy distinct 'territories' also in the interphase, i.e. between cell divisions. In an analogy of the Bauhaus principle that "form follows function" we believe that analyzing in which form DNA is organized in these territories will help us to understand genomic function. We use computer models - Monte Carlo and Brownian dynamics simulations - to develop plausible proposals for the structure of the interphase genome and compare them to experimental data. In the work presented here, we simulate interphase chromosomes for different folding morphologies of the chromatin fiber which is organized into loops of 100kbp to 3 Mbp that can be interconnected in various ways. The backbone of the fiber is described by a wormlike-chain polymer whose diameter and stiffness can be estimated from independent measurements. The implementation describes this polymer as a segmented chain with 3000 to 20000 segments for chromosome 15 depending on the phase of the simulation. The modeling is performed on a parallel computer (IBM SP2 with 80 nodes). We also determine genomic marker distributions within the Prader-Willi-Region on chromosome 15q11.2-13.3. For these measurements we use a fluorescence in situ hybridisation method (in collaboration with I. Solovai, J. Crai and T. Cremer, Munich, FRG) conserving the structure of the nucleus. As probes we use 10 kbp long lambda clones (Prof. B. Horsthemke, Essen, FRG) covering genomic marker distances between 8 kbp and 250 kbp. The markers are detected with confocal and standing wavefield light microscopes (in collaboration with J.Rauch, J. Bradl, C. Cremer and E.Stelzer, both Heidelberg, FRG) and using special image reconstruction methods developed solely for this purpose (developed by R. Eils. and W. Jaeger, Heidelberg, FRG).
Best agreement between simulations and experiments is reached for a Multi-Loop-Subcompartment model with a loop size of 126 kbp which are forming rosetts and are linked by a chromatin linker of again 126 kbp. We also hypothesize a different folding structure for maternal versus paternal chromosome 15. In simulations of whole cell nuclei this modell also leads to distinct chromosome territories and subcompartments. A fractal analysis of the simulations leads to multifractal behavior in good agreement with predictions drawn from porous network research.

The work is part of the Heidelberg 3D Human Genome Study Group, which is part of the German Human Genome Project.

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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, autofluorescent proteins, CFP, GFP, YFP, DsRed, fusionprotein, in vivo labelling.

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