Diffusion and transport in the human interphase cell nucleus
FCS experiments compared to simulations

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FCS experiments

Basics

Mean square displacement (MSD) of a free Brownian particle:

$$\left\langle \mathbf{r}(t)^2 \right\rangle = 6D_0 t, \qquad D_0 = \frac{kT}{6\pi\eta r_h} \qquad (1), (2)$$

(D_0: diffusion coefficient, r_h: hydrodynamic radius, η: viscosity).
Mean square displacement in the presence of obstacles:

$$\left\langle \mathbf{r}(t)^2 \right\rangle = 6D(t)\, t \;\mu\; t^{2/d_w} \qquad (3)$$

This behaviour is called obstructed diffusion. The anomaly parameter d_w characterizes the time-dependent diffusion coefficient $D(t)$ and equals 2 for free diffusion. It increases with increasing obstacle concentration and depends strongly on geometric properties like the obstacle size or the fractal dimensions of the distribution.
If the obstacles form cages, dead-ends, or cavities, molecules can be trapped, resulting in an apparently slowly and a freely diffusing fraction of molecules.

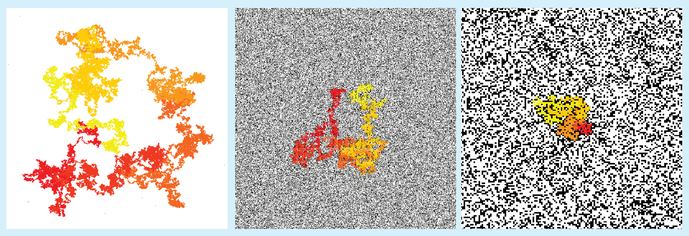
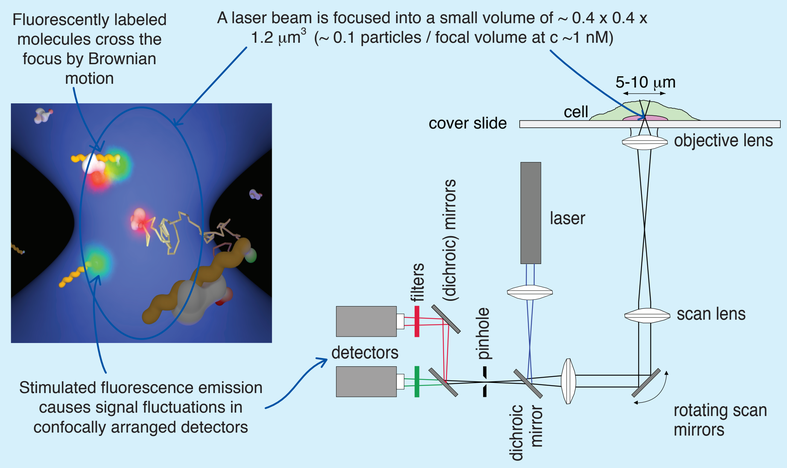


Fig. 1: Simulated random walk of 10^6 steps on an empty rectangular 1500x1500 site lattice (left); the path colour is changed from red to yellow with time. In the presence of statistically distributed obstacles with a density of 35% and a size of 2x2 (middle) or 8x8 sites (right), respectively, the area covered by the random walk gets smaller and shows different "compactnesses" for different obstacle geometries.

Fluorescence correlation spectroscopy



A computer calculates the autocorrelation function (ACF) of the detector signals. The concentration and the diffusion coefficient of the molecules can be derived.
The excitation and detection path are coupled via a scanning unit into a conventional inverted microscope (Olympus IX70), providing a diffraction limited focus and a corresponding spatial resolution. The compact FCS/scanning module can be easily attached to the video port of the microscope and shows a high optical and mechanical stability.

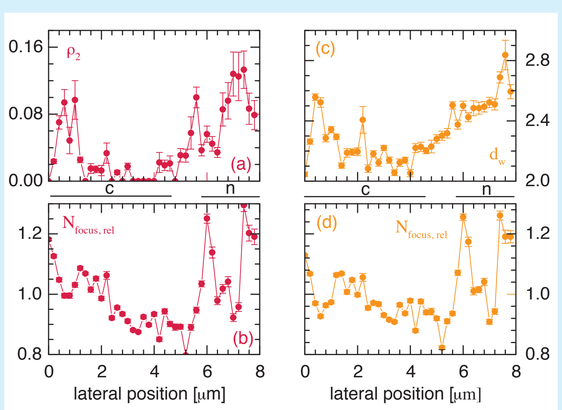
Diffusion scans

		two components	obstructed diffusion	number of cells
AT-1:	$D_{\text{monomer, aq.}}/D_{\text{monomer, cell}}$	5.3 0.6	5.5 0.6	9
	$D_{\text{monomer, cell}}/D_{\text{fus.prot., cell}}$	1.4 0.2	1.5 0.4	6
COS-7:	$D_{\text{monomer, aq.}}/D_{\text{monomer, cell}}$	4.7 0.5	4.5 0.7	8
	$D_{\text{monomer, cell}}/D_{\text{fus.prot., cell}}$	1.2 0.1	1.3 0.3	4

Table 1: The diffusion coefficient and therefore the viscosity sensed by eGFP and the fusion protein is about 5 times higher than in water in AT-1 cells and in COS-7 cells. This holds for nuclei as well as for the cytoplasm, implying that the nuclear "solvent" is similar to the cytosol.

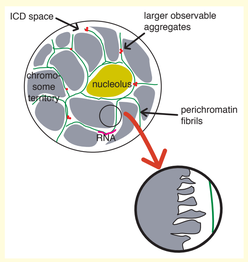
From FCS data along straight lines in AT-1 and COS-7 cells expressing eGFP or an eGFP-b-galactosidase fusion protein (Fig. 2) we obtain a deviation from ideal diffusion especially in the nucleus.
Interpretation as a fast moving fraction everywhere in the cell and a slower one mainly in the nucleus: only an inhomogeneous chromatin distribution with high local densities leads to trapping and subsequent observation of two distinct fractions.
Applying the obstructed diffusion model: diffusion obstruction is found mainly in the nucleus. Even low obstacle densities lead to a remarkable deviation from free diffusion.

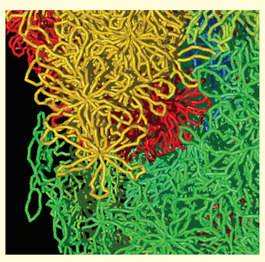
Fig. 2: FCS scan through a COS-7 cell expressing the fusion protein: (a) fraction of a slow component and (b) relative number of molecules, found with the two component free diffusion model; (c) anomaly parameter and (d) relative molecule number from the obstructed diffusion model, as a function of the position in the cell ("c" - cytoplasm, "n" - nucleus).



Introduction

Despite the succesful linear sequencing of the human genome the three-dimensional arrangement of chromatin, functional, and structural components is still largely unknown. Molecular transport and diffusion are important for processes like gene regulation, replication, or repair and are vitally influenced by the structure. With a comparison between fluorescence correlation spectroscopy (FCS) experiments and simulations we show here an interdisciplinary approach for the understanding of transport and diffusion properties in the human interphase cell nucleus.





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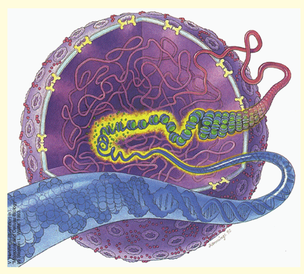


Conclusion

FCS in combination with a scanning device is a suitable tool to study the diffusion characteristics of fluorescent proteins in living cell nuclei with high spatial resolution. Computer simulations of the three-dimensional organization of the human interphase nucleus allows a detailed test of theoretical models in comparison to experiments. Diffusion and transport in the nucleus are most appropriately described with the concept of obstructed diffusion. A large volume fraction of the nucleus seems to contain a cytosol-like liquid with an apparent viscosity 5 times higher than in water. The geometry of particles and structure as well as their interactions influence the mobilities in terms of speed and spatial coverage. A considerable amount of genomic sites is accessible for not too large particles. FCS experiments and simulations based on the polymer model are in a good agreement. Using recently developed in vivo chromatin markers, a detailed study of mobility vs. structure is subject of current work.

Diffusion vs. structure

The diffusion of particles in living interphase nuclei depends on the local structure. The development of in vivo chromatin markers allows to investigate this relation using FCS. The correlation between diffusion obstruction and structure vanishes for small particles and probably increases with increasing particle size (Fig. 2, 3, 7).

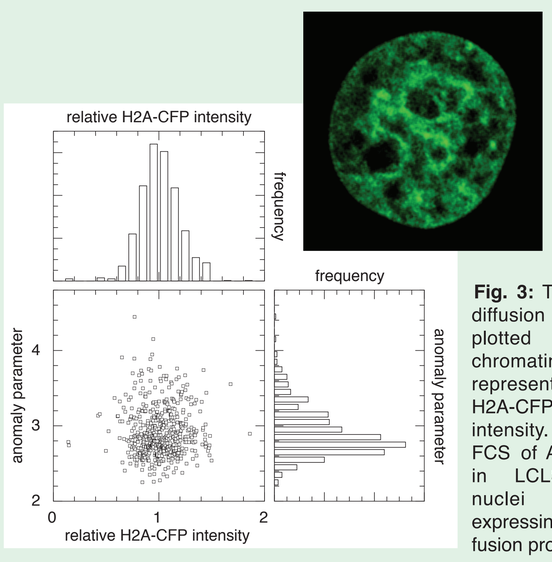


Fig. 3: The degree of diffusion obstruction plotted against the chromatin density, represented by the H2A-CFP fluorescence intensity. Data from FCS of Alexa568 dye in LCLS103H cell nuclei stably expressing a H2A-CFP fusion protein.

Simulations

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. 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 rosette-like MLS model leads to clearly distinct functional and dynamic subcompartments in agreement with experiments (Fig. 4B) in contrast to the RW/GL models where big loops are intermingling freely and featureless (Fig. 4C & 4D).

Fig. 4A: Starting configur-ation with the form and size of a metaphase chromosome.

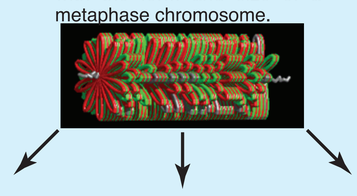


Fig. 4B: MLS model with 126 kbp loops & linkers.

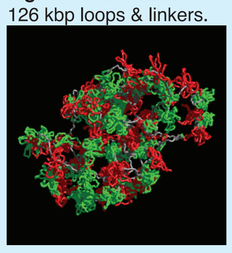


Fig. 4C: RW/GL model with 126 kbp loops.

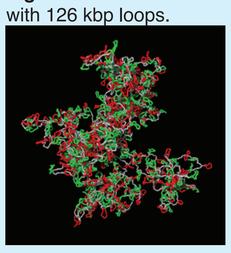


Fig. 4D: RW/GL model with 5 Mbp loops.

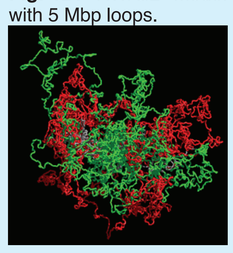
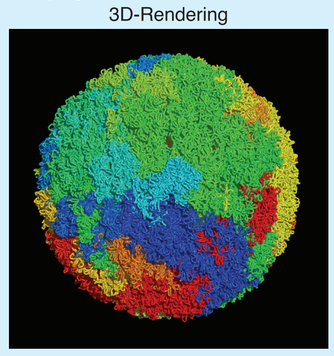
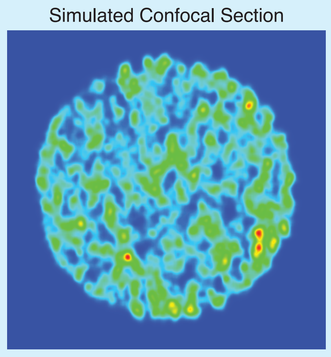


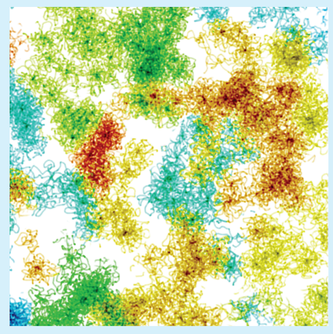
Fig. 5A - 5D: Simulation of a human interphase nucleus containing all 46 chromosomes with 1,200,000 polymer segments. The MLS-model leads to the formation of distinct and non-overlaping chromosome territories.

3D-Rendering

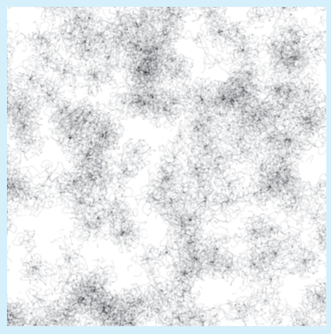


Simulated Confocal Section





Simulated EM-FISH Section



Simulated EM Section

The diffusion of spherical particles with radius r_h in a nucleus is simulated using Brownian Dynamics methods. The mean square displacement of the particles depends on r_h, the radius of the nucleus, i.e. the obstacle concentration, and also critically on the interaction between particles and structure (Fig. 6 & 7). The results agree with theoretical expectations as well as with FCS experiments (Table 1).

Fig. 6: Comparison of mean square displacements of particles with different radius r_h in nuclei with 3 m (g, y) and 6 m (r,b) radius and low (r,g) and high (b,y) interaction.

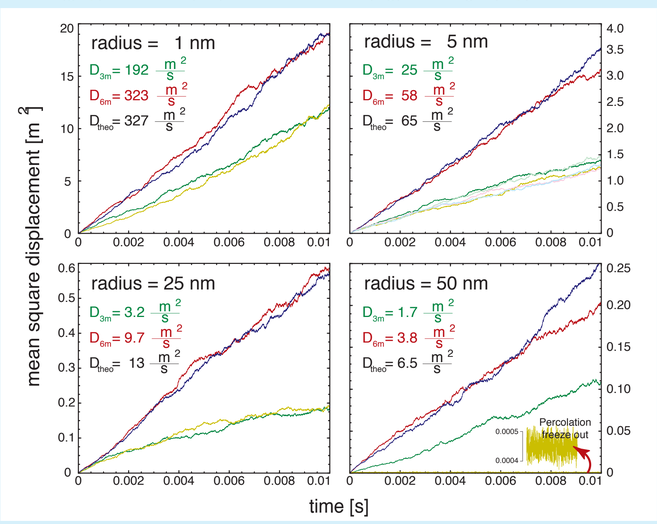
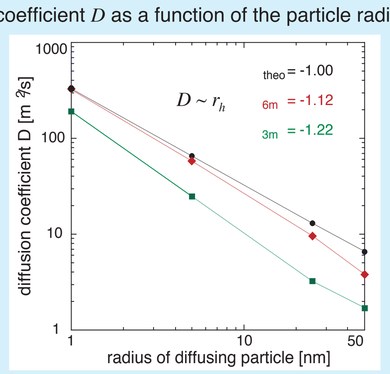


Fig. 7: Apparent diffusion coefficient D as a function of the particle radius r_h for different nuclear radii.



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FCS Experiments compared to Simulations

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Abstract

Despite the succesful linear sequencing of the human genome the three-dimensional arrangement of chromatin, functional, and structural components is still largely unknown. Molecular transport and diffusion are important for processes like gene regulation, replication, or repair and are vitally influenced by the structure. With a comparison between fluorescence correlation spectroscopy (FCS) experiments and simulations we show here an interdisciplinary approach for the understanding of transport and diffusion properties in the human interphase cell nucleus.

For a long time the interphase nucleus has been viewed as a 'spaghetti soup' of DNA without much internal structure, except during cell division. Only recently has it become apparent that chromosomes occupy distinct 'territories' also in interphase. Two models for the detailed folding of the 30 nm chromatin fibre within these territories are under debate: In the Random-Walk/Giant-Loop-model big loops of 3 to 5 Mbp are attached to a non-DNA backbone. In the Multi-Loop-Subcompartment (MLS) model loops of around 120 kbp are forming rosettes which are also interconnected by the chromatin fibre. Here we show with a comparison between simulations and experiments an interdisciplinary approach leading to a determination of the three-dimensional organization of the human genome: For the predictions of experiments various models of human interphase chromosomes and the whole cell nucleus were simulated with Monte Carlo and Brownian Dynamics methods. Only the MLS-model leads to the formation of non-overlapping chromosome territories and distinct functional and dynamic subcompartments in agreement with experiments. Fluorescence in situ hybridization is used for the specific marking of chromosome arms and pairs of small chromosomal DNA regions. The labelling is visualized with confocal laser scanning microscopy followed by image reconstruction procedures. Chromosome arms show only small overlap and globular substructures as predicted by the MLS-model. The spatial distances between pairs of genomic markers as function of their genomic separation result in a MLS-model with loop and linker sizes around 126 kbp. With the development of GFP-fusion-proteins it is possible to study the chromatin distribution and dynamics resulting from cell cycle, treatment by chemicals or radiation in vivo. The chromatin distributions are similar to those found in the simulation of whole cell nuclei of the MLS-model. 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. Fractal analysis of the simulations reveal the multi-fractality of chromosomes. First fractal analysis of chromatin distributions *in vivo* result in significant differences for different morphologies and might favour a MLS-model-like chromatin distribution. Simulations of fragment distributions based on double strand breakage after carbon-ion irradiation differ in different models. Here again a comparison with experiments favours a MLS-model.

FCS in combination with a scanning device is a suitable tool to study the diffusion characteristics of fluorescent proteins in living cell nuclei with high spatial resolution. Computer simulations of the three-dimensional organization of the human interphase nucleus allows a detailed test of theoretical models in comparison to

experiments. Diffusion and transport in the nucleus are most appropriately described with the concept of obstructed diffusion. A large volume fraction of the nucleus seems to contain a cytosol-like liquid with an apparent viscosity 5 times higher than in water. The geometry of particles and structure as well as their interactions influence the mobilities in terms of speed and spatial coverage. A considerable amount of genomic sites is accessible for not too large particles. FCS experiments and simulations based on the polymer model are in a good agreement. Using recently developed in vivo chromatin markers, a detailed study of mobility vs. structure is subject of current work.

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Keywords:

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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