**FCS experiments**

### Basics

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

\[
\langle r(t)^2 \rangle = 6Dt + \text{bias}_t \quad \text{(2)}
\]

(\(D\), diffusion coefficient, \(r_t\) hydrodynamic radius, \(\nu_t\) viscosity).

Mean square displacement in the presence of obstacles:

\[
\langle r(t)^2 \rangle = 6D_l(t) + \text{bias}_t \quad \text{(3)}
\]

This behaviour is called obstructed diffusion. The anomaly parameter \(\nu_r\) 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.

### Fluorescence correlation spectroscopy

Fluorescence correlation spectroscopy (FCS) is a powerful tool for studying the concentration and the diffusion coefficient of the molecules in a cell. The correlation between diffusion and structure 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 there is an interdisciplinary approach for the understanding of transport and diffusion properties in the human interphase cell nucleus.

### Introduction

Despite the successful 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 there is an interdisciplinary approach for the understanding of transport and diffusion properties in the human interphase cell nucleus.

### Table 1: Diffusion obstruction

<table>
<thead>
<tr>
<th>Model</th>
<th>Diffusion obstruction number of cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>AT-1:</td>
<td>5.25 0.6 5.5 6.6 6</td>
</tr>
<tr>
<td>COS-7:</td>
<td>4.75 0.5 4.9 6.7 8</td>
</tr>
<tr>
<td>COS-7:</td>
<td>1.2 0.1 1.3 0.3 4</td>
</tr>
</tbody>
</table>

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### Diffusion vs. structure

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

### 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 cytoplasm-like liquid with an apparent velocity 5 times higher than in water. The geometry of particles and structure as well as their interaction effects influence the motion in the cell nucleus. A considerable amount of geometric sites is accessible for not too large particles. FCS experiments and simulations model are in good agreement. Using recently developed in vivo chromatin markers, a detailed study of motility vs. structure is subject of current work.

### Simulations

For the prediction of experiments we simulated various models of human interphase chromosome 15 with Monte Carlo and Brownian Dynamic methods. The chromatin fiber was modeled as a flexible polymer. Only stretching, bending and excluded volume interactions are considered. Chromosomes are further fibrated by a spherical potential representing the surrounding chromosomes or the nuclear membrane. Computer simulations of the human interphase cell nucleus allow a detailed test of theoretical models in agreement with experiments (Fig. 4D & 40).
Literature


Diffusion and Transport in the Human Interphase Cell Nucleus

- FCS Experiments compared to Simulations

Wachsmuth, M., Knoch, T. A., Münk, C. & Langowski, J.

Poster presentation of Scientific Studies from Diploma- and PhD- Students of the German Cancer Research Centre (DKFZ), Heidelberg, Germany, 14th - 18th January, 2001.

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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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.

Literature References


