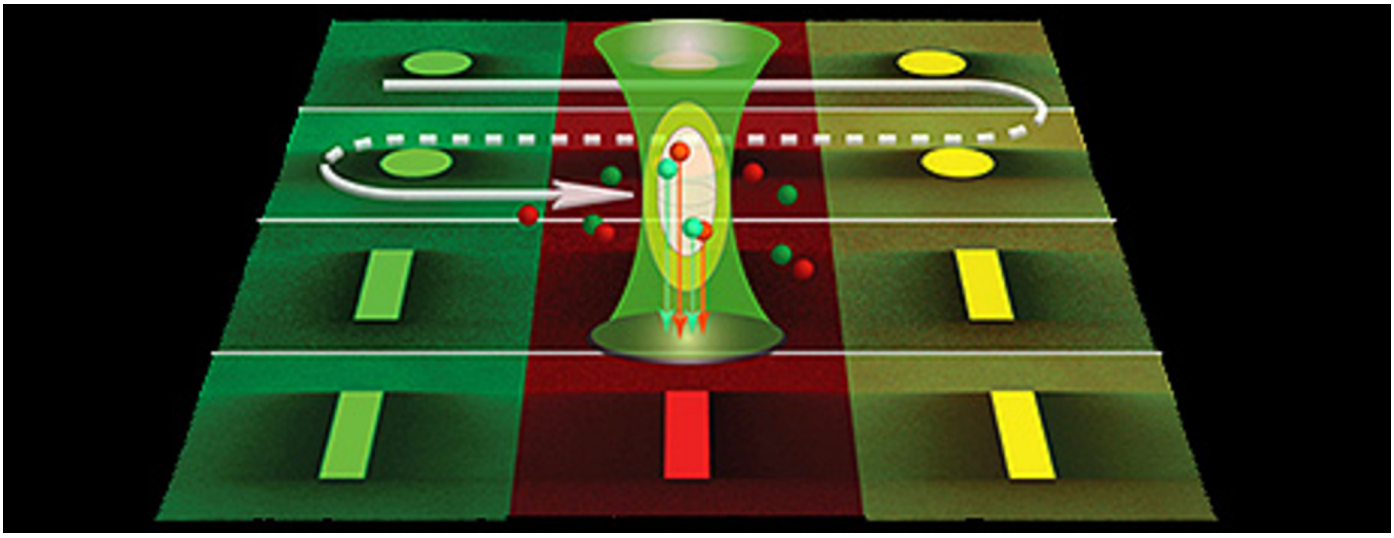
Website address:
https://www.gesundheitsindustrie-bw.de/en/article/press-release/need-for-speed-high-speed-measurements-of-molecular-motion-in-the-cell-nucleus

Need for Speed: High-speed Measurements of Molecular Motion in the Cell Nucleus

They were able to measure the binding of highly specialised protein complexes that specifically change the spatial structure of the genetic information, thereby controlling the readout of the DNA information. The work of Dr. Karsten Rippe and his team was carried out at the BioQuant Center of Heidelberg University and the German Cancer Research Center. Their research has demonstrated that the positioning of nucleosomes – complexes of DNA and specialised proteins – is a precisely regulated molecular process. Aberrant regulation can be linked to several types of cancer. The results of these studies were published in the journal PNAS.



Scheme of microscopy images after bleaching the fluorescence in a circular or rectangular region. The dark "shadows" represent the bleached proteins that moved as the image was being recorded.

In the human genome, the DNA strands are wrapped around specific packaging proteins, the histones. Located between these complexes, called nucleosomes, are histone-free DNA sequences that connect the nucleosomes, much like a pearl necklace. "Activating a gene requires freely accessible DNA. If the corresponding DNA is occluded in the nucleosome, the gene is often turned off", explains Dr. Rippe. "Hence, the nucleosome positions determine the readout pattern of the DNA sequence. The free DNA between two nucleosomes is more easily accessible than the DNA sequences in a nucleosome." Molecular machines called chromatin remodelers can use energy to move nucleosomes along the DNA chain. Thus they establish the readout pattern that, along with other factors, determines the active DNA programme of the cell. Rippe's team of scientists are using fluorescence microscopy to investigate how the chromatin remodelers control the readout of the genetic information. With it they were able to measure that most of the approx. one million chromatin remodelers in the human cell transiently bind to nucleosomes to test whether all the approx. 30 million nucleosomes are at the right position. A new way of measurement was needed to understand how these molecular machines work. "We had to record short binding events at a resolution of 1/1000 of a second and at the same time detect the rare events with a binding time of several seconds or even minutes", says Karsten Rippe. Doctoral student Fabian Erdel came up with an idea that led to "Pixel-wise Photobleaching Profile Evolution Analysis", or 3PEA, which can be used to take such measurements in living cells. In his experiments, Fabian Erdel used a laser beam to extinguish the artificial fluorescent tag attached to the chromatin remodelers. He noticed that the "bleached" proteins produced a "shadow" when they moved while the image was being recorded. The shape of this shadow depended on how much the movement of the chromatin remodelers slowed down due to binding to nucleosomes. "It was not easy calculating duration times of binding from the shadow image, but it was worth the effort. Our method has exciting new applications because we can use it to measure the binding of proteins in living cells very quickly and precisely", remarks Fabian Erdel. Using 3PEA measurements, the researchers

demonstrated that an individual chromatin remodeler travels through the entire cell nucleus within a single second testing more than 300 nucleosomes – mostly without becoming active. Only occasionally the molecular machine would bind to a nucleosome for several seconds or even minutes, causing it to shift position on the DNA. Dr. Rippe and his team next want to decode signals that activate the chromatin remodelers at certain locations on the genome.

Original publication::
F. Erdel, K. Rippe: Quantifying transient binding of ISWI chromatin remodelers in living cells by pixel-wise photobleaching profile evolution analysis, PNAS, 20 November 2012, vol. 109, no. 47, E3221-3230 (online 5 November 2012, doi:10.1073/pnas.1209579109).

More information:
Dr. Karsten Rippe
BioQuant
University of Heidelberg
Phone: +49 (0)6221/ 54 - 51376
E-mail: karsten.rippe(at)bioquant.uni-heidelberg.de

Press release

14-Dec-2012
Source: Universität Heidelberg (03.12.2012)

UNIVERSITÄT
HEIDELBERG
ZUKUNFT
SEIT 1386