Intracellular mobility of autofluorescent proteins: a tool for understanding the polymer network structure of the genome

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The three-dimensional folding of the genome has major implication for gene regulation and cell differentiation. In order to understand its structure, we follow a multidisciplinary approach using fluorescence techniques for studying the mobility of nuclear proteins, and a set of computer models that describe the genome as a flexible polymer.
A newly developed technique, fluorescence fluctuation microscopy (FFM) allows us to observe the mobility and concentration of fluorescent probe molecules in living cells. FFM is a combination of fluorescence correlation spectroscopy (FCS) and continuous fluorescence microphotolysis (CFM). In our FFM instrument a point-addressable confocal scanner is coupled to an FCS module, such that individual points in a confocal image can be accessed with a precision of less than 50 nm. By combining photobleaching measurements with FCS, mobilities, free and bound fractions of fluorescent probes such as GFP fusion proteins can be measured at different positions in the cell. Comparing these results with simulations of the Brownian motion of molecules in the chromatin network allows us to verify our models of interphase chromosome structure and dynamics. An important result is that most of the interphase chromatin is accessible to medium-size protein complexes; with the exception of the nucleolus, no very dense regions exist in the nucleus that would obstruct diffusion completely.