1. BACKGROUND. The angles of entry and exit of DNA from the nucleosome and the length of linker DNA between nucleosomes are determined by its interaction with histone H1. Owing to this property H1 participates, by maintenance of higher order chromatin structures, in regulation of gene expression and DNA replication. The pool of histone H1 localized on DNA undergoes rapid exchange with its counterpart in nucleoplasm. Moreover, subtypes of histone H1 are reported to differ in their preference for active (euchromatin) and inactive (heterochromatin) regions in cell nuclei. Nonetheless, little is known about relationship between the mode and dynamics of histone H1 binding to DNA and the chromatin structure in living cells.

2. METHODS. We applied confocal microscopy and raster image correlation spectroscopy (RICS) to study H1 mobility in different regions of cell nuclei. This task was executed by calculation of image autocorrelation patterns locally, using moving window approach. We characterized the local H1 mobility using diffusion coefficients of the mobile fractions of the histone and the respective binding times of its immobile fractions. We correlated this H1 mobility characteristics with local chromatin structure, determined using image moments (Kravtchouk) and texture classification. Furthermore, we validated these data with point-wise measurements using fluorescence correlation spectroscopy (FCS).

3. RESULTS. The RICS measurements demonstrate that H1 dynamics is primarily determined by its binding to chromatin, with the characteristic time of from 8.5 to 9.5 ms. However, in certain regions of nuclei the presence of mobile fraction of H1 was detectable, in addition to the bound fraction. The mobility of the former was characterized by diffusion coefficient from 0.2 to 0.6 \( \mu m^2/s \). The regions where the mobile fraction of H1 is present are characterized by uniform chromatin texture. On the other hand the regions where only immobile (bound) fraction of H1 is present correspond to blocks of condensed chromatin (i.e. non-uniform texture). Moreover, the mobility of H1 may occur at several different time scales (corresponding to multiple diffusion coefficients). This notion, as well as the presence of mobile and immobile fractions are confirmed with FCS.