Biophysical Journal, Volume *112*

Supplemental Information

Tracking Multiple Genomic Elements Using Correlative CRISPR Imaging and Sequential DNA FISH

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Supplementary Material

Supplementary Data

Excel spreadsheet listing oligo array sequences used to generate FISH probes for loci NR1 and NR2 respectively.

Supplementary Video S1

The dynamics of genomic loci corresponds to Figure 4a-d. The last frame shows the tracked trajectories.

Supplementary Figures

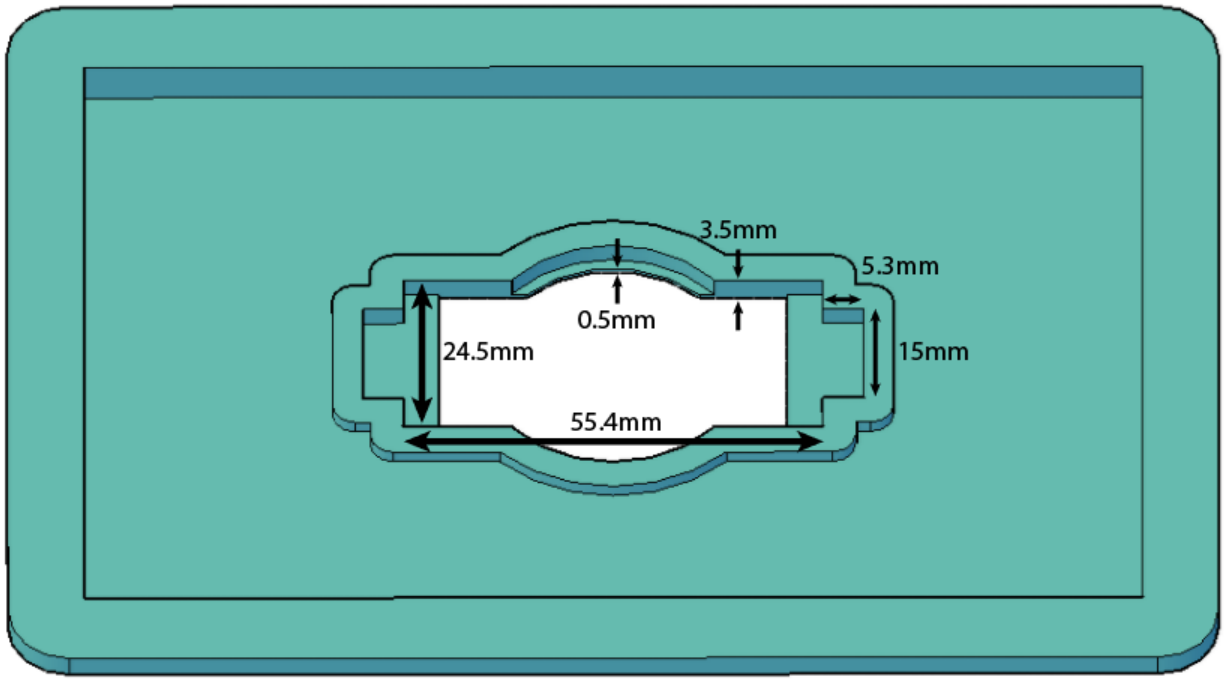

Figure S1 Schematic of the 3D-printed stage adaptor to fit the 8-well imaging chamber (Lab-Tek) onto the microscope stage.

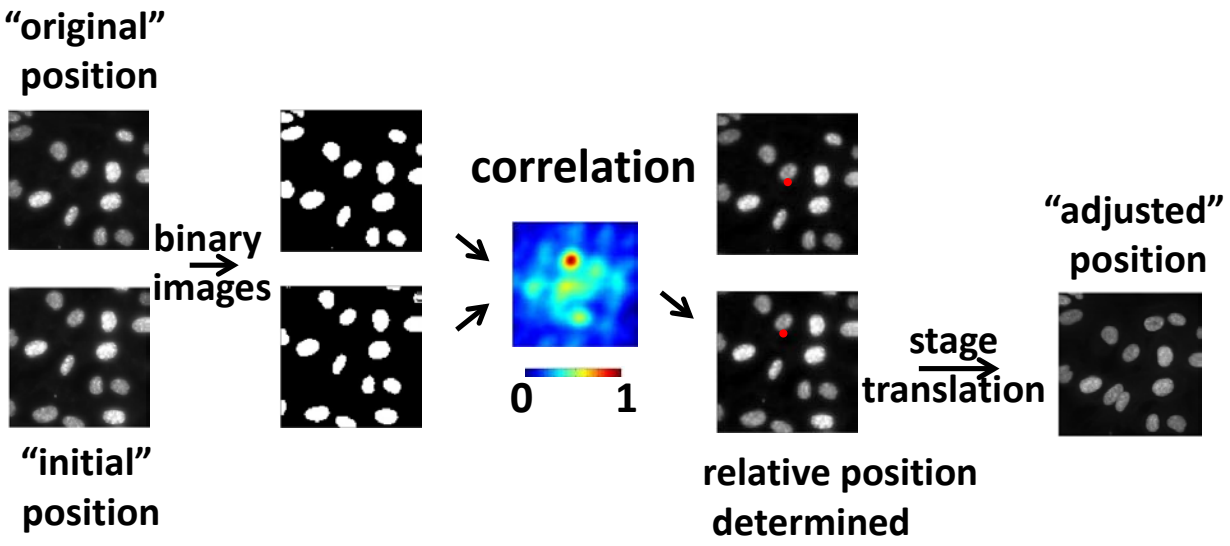

Figure S2 Stage registration algorithm. See methods in main text for details.

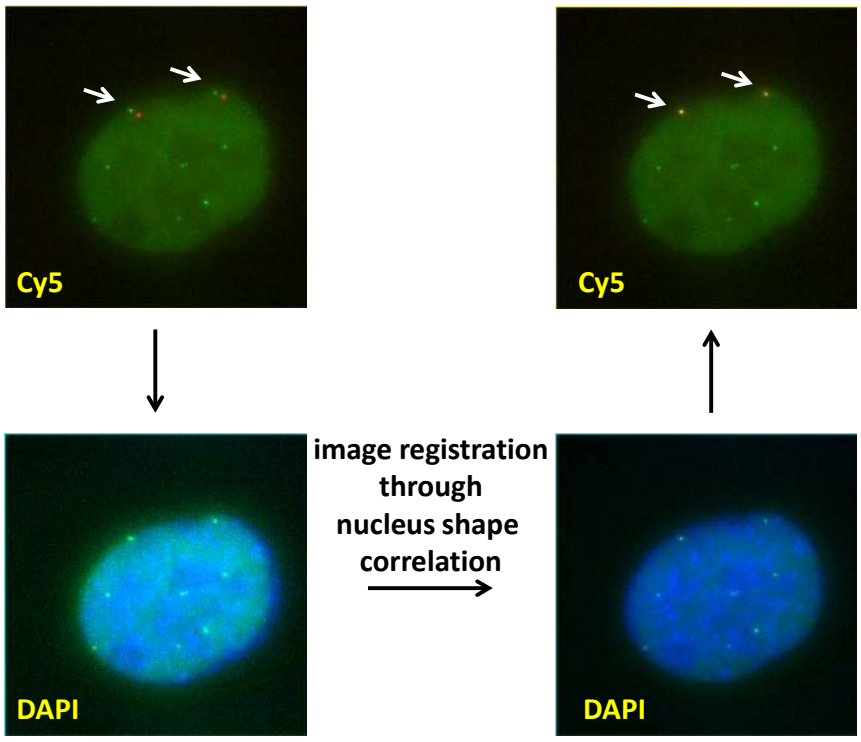

Figure S3 Refined image registration algorithm to register loci features based on nuclear shape. See methods in main text for details. Field of view is 30 μm × 30 μm.

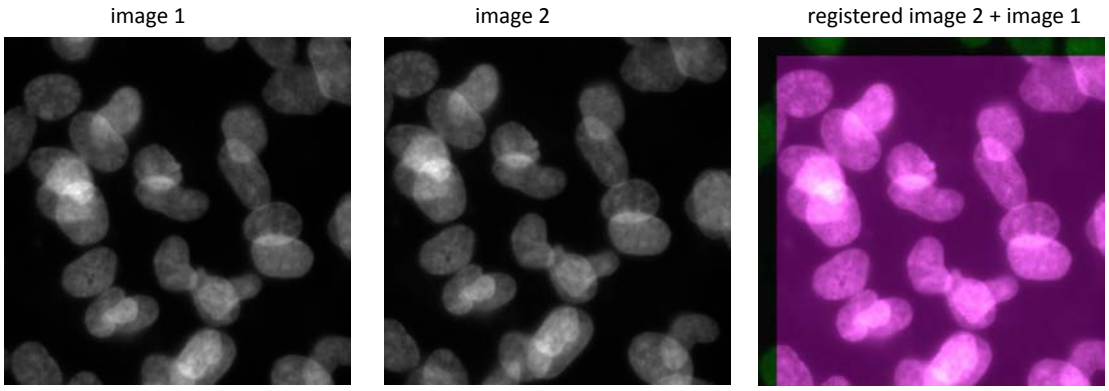

Figure S4 The image registration algorithm remains robust when features overlap at a high density. The overlapping feature is generated by overlaying two less dense images.

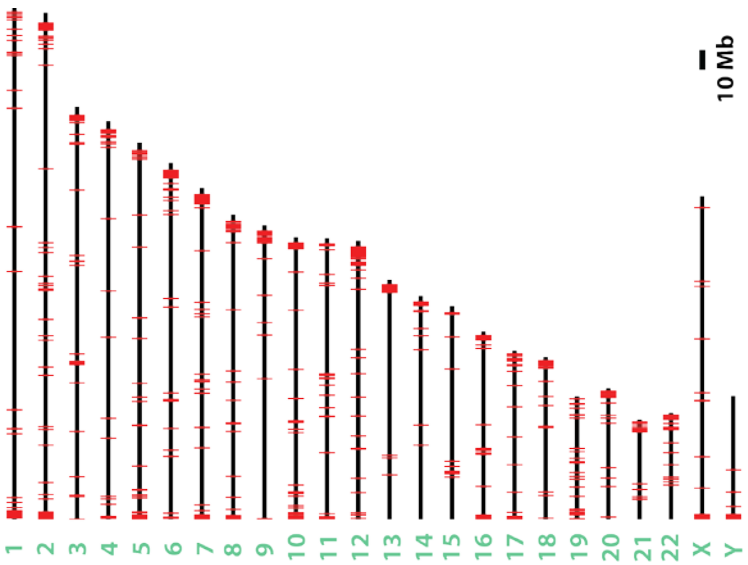

Figure S5 Human genome is abundant with tandemly repetitive sequences (TRS) potentially compatible with CRISPR imaging, about 100 loci per chromosome. The red bars denote the positions of these TRS site on the 24 human chromosomes.

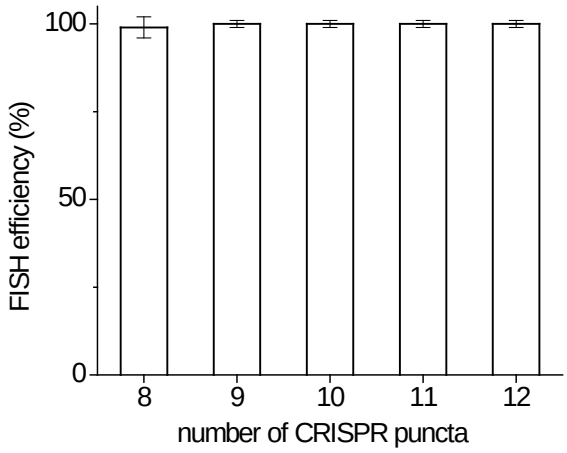

Figure S6 The efficiency of FISH staining is consistently almost 100%. The efficiency is calculated as the ratio of observed to expected number of FISH puncta. Error bars denote standard deviation.

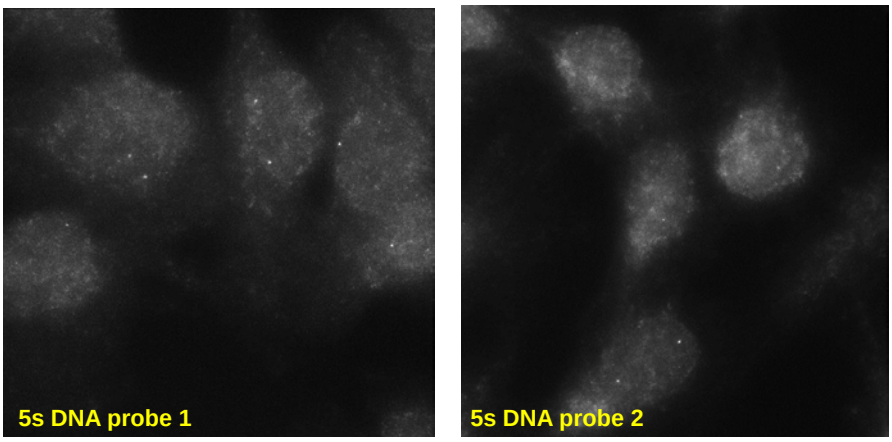

Figure S7 Examples of two oligo sequences staining human 5s DNA which has a copy number ranging from 35 to 175.

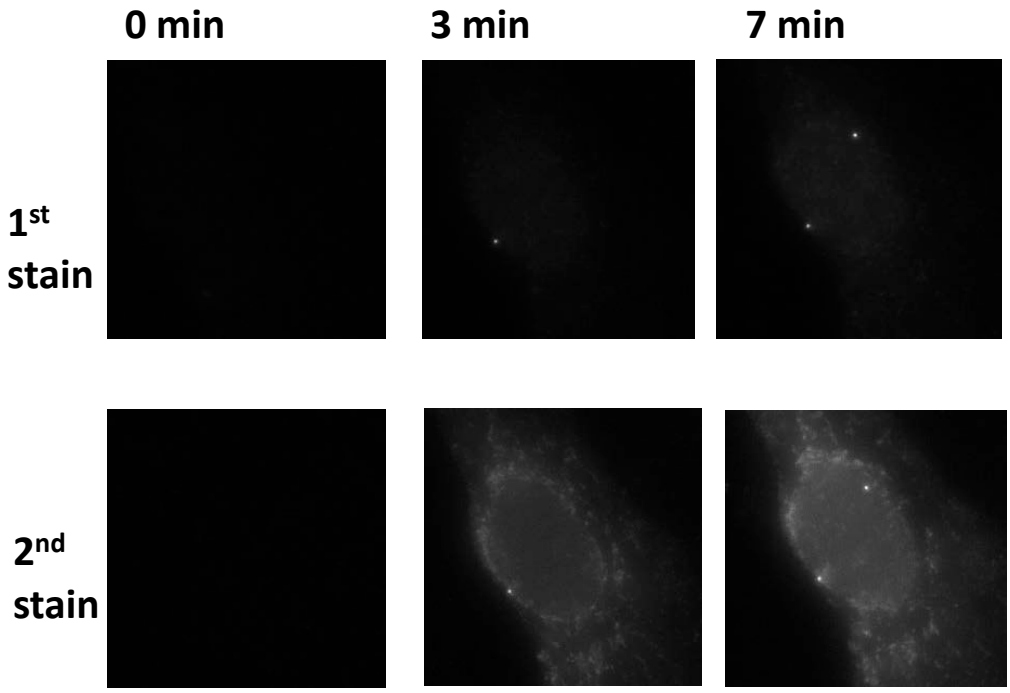

Figure S8 Heating duration affects the accessibility of genome. The same cell shows 0, 1, and 2 spots after heating at 80C for 0 min, 3 min, and 7 min respectively. Staining time is 2 min. A second stain after washing and removing the bound probes at each time point confirms the trend.

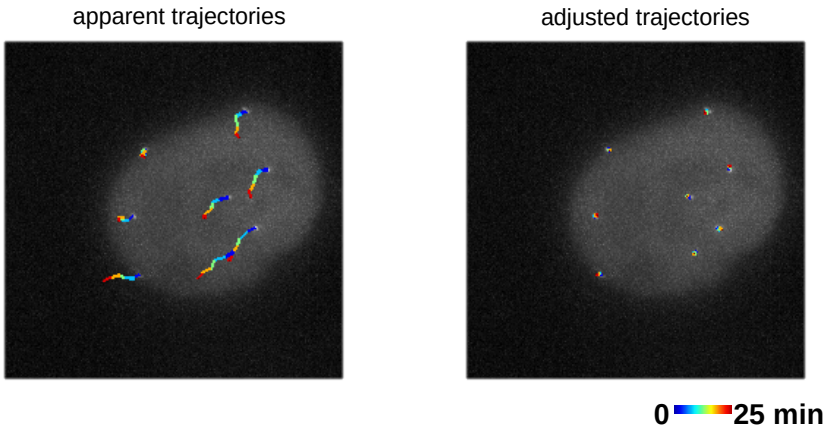

Figure S9 (left) Apparent trajectories of loci reflecting the absolute displacement overlaid with live image at t = 0 min. (Right) Adjusted trajectories of loci reflecting the relative displacement after subtracting the global movement of nucleus overlaid with live image at t = 0 min. The relative displacement between loci is reduced in amplitude and randomized in direction compared to absolute displacement. The nucleus is the same as in Figure 4.

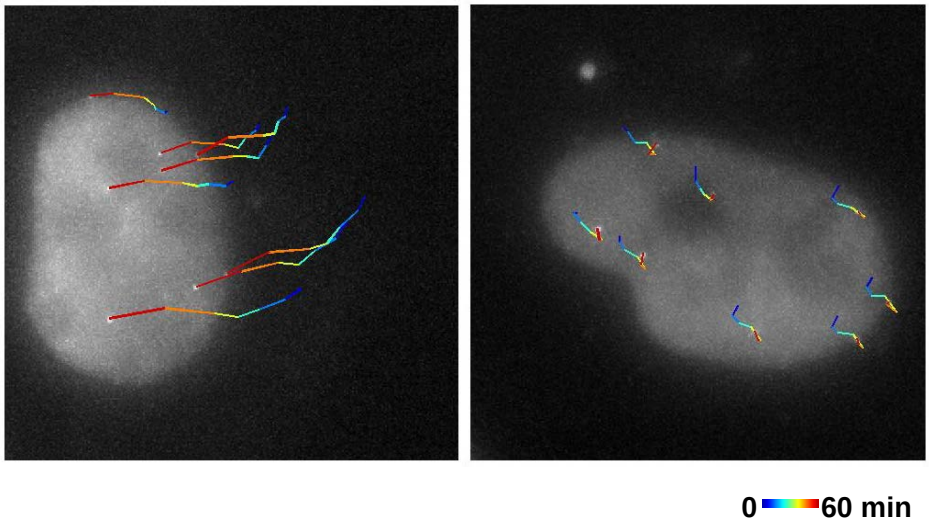

Figure S10 Two representative trajectories overlaid with the nuclei at t = 60 min. Global movement of nuclei and stable relative position of loci is a common feature observed in live-cell imaging.

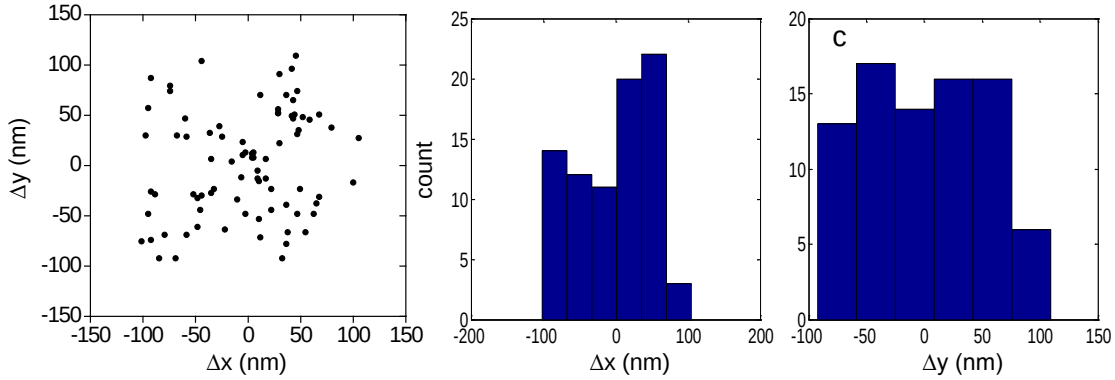

Figure S11 (a) The scatter plot of loci position in fixed images compared to live-cell position set at the origin. (b-c) The histogram showing the error distribution in registration of loci position. The RMS error is ~52 nm.

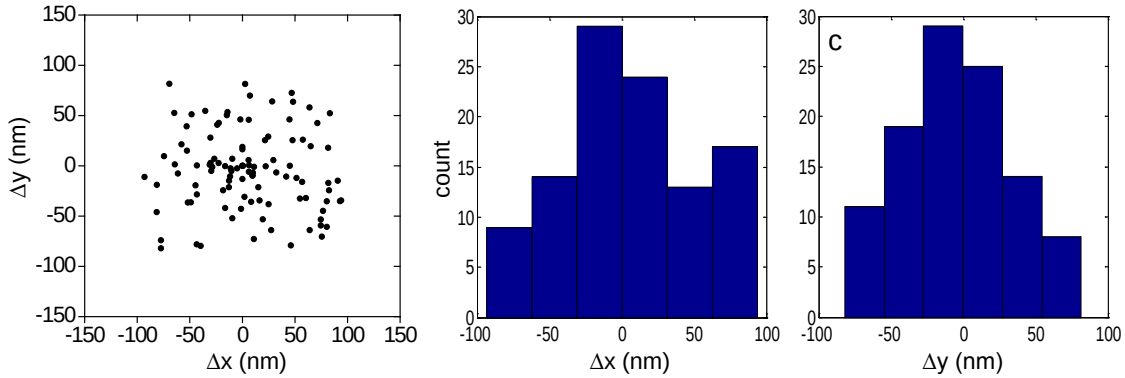

Figure S12 (a) The scatter plot of loci position between two sequential DNA FISH hybridization rounds with the loci position in the first round set at the origin. (b-c) The histogram showing the error distribution in registration of loci position. The RMS error is ~43 nm.

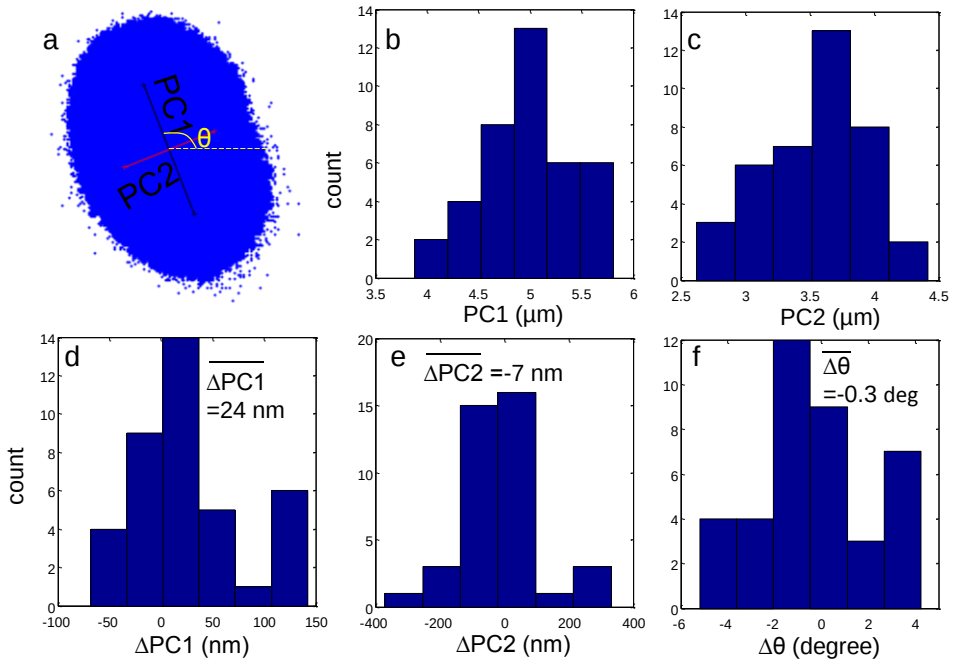

Figure S13 (a) Principle component analysis of nuclear shape. The first two principal components correspond to the long and short axis of nuclei. θ characterize the nuclear orientation. (b-c) The histogram of long and short axis distribution in live-cell imaging respectively. (d-f) The histogram of the change between live-cell images and fixed images in long axis, short axis, and angle of rotation, respectively.