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Live cell microscopy approaches to dissect chromatin dynamics in 3D at high temporal resolution

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Higher-order chromatin structures are multi-dimensional and dynamically adapting to nuclear function and complexity, at various time scales. Imaging chromatin and nuclear organisation in individual cells is necessary to understand how chromatin folds and behaves.

We are using two new approaches to study chromosome dynamics in 3D at high temporal resolution in the yeast S. cerevisiae. First, we visualize three independent chromosomal loci simultaneously. We created vectors to integrate repetitions of lambda repressor binding sites to which fluorescent fusion proteins between the lambda repressor and YFP can bind. Coupled to the well established lacOp and tetOp system we validate its use to determine the 3D positioning and dynamics of three loci on chromosome 3 of S.c.

Second, extremely short acquisition times of the movement of labelled loci enable quantification of the flexibility of the chromatin fiber with respect to the position of the locus along the chromosome and its position within the nuclear volume. Assaying chromatin dynamics with ms time intervals and an excellent signal to noise ratio provides a positional precision of 20 nm and allows determination of the spontaneous diffusion of specific sites along the chromosome. Our studies provide new structural insights of chromatin organization.