# High-accuracy determination of chromatin and the transcription machinery motions using super-resolution imaging

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Gene expression is a highly dynamic and cell type specific process regulating fundamental functions during growth and development. Control of gene expression mostly occurs at the transcription stage, based on modifications of chromatin structure and transcription factors (TF) along with RNA polymerase II (RNA pol2) dynamics. These dynamic changes were described in fixed and in living cells by super-resolution wide-field fluorescence microscopy techniques [1, 2]. However, quantification of the dynamic properties of these biological processes at short time sampling and nanoscale resolution in living eurokaryotic cells is still missing. In particular, the orientation of chromatin and RNA Pol II during the early transcription stages is unclear. For this purpose, we developed a new method combining super resolution imaging (PALM) and optical flow methods [3-5]. Differential methods for estimating velocity fields at sub-pixel accuracy, based on partial derivatives of the image signal [5, 6], were applied to measure the direction and the magnitude of local movements of fluorescently labeled proteins simultaneously across the entire nucleus comparing pairs of super resolved images. Moreover, we were able to calculate correlations between the reported local chromatin, TF and RNA pol II displacements at different time intervals. These measurements increase our understanding of the coordinated motion of the transcription machinery and chromatin.

References

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